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URINARY AND FECAL EXCRETION OF GOLD BY RATS GIVEN SODIUM AUROTHIOMALATE

by

Ⓒ JEANETTE WAKARUK

A THESIS

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ABSTRACT

The excretion of gold in urine and feces of Sprague Dawley rats has been examined after administration of radioactive ^{198}Au-labelled sodium aurothiomalate (ATM). Single doses of ^{198}Au-ATM (0.5 mg and 1.0 mg Au/kg,im) were injected into rats and the urine and feces collected at 24 ± 4 hr intervals for 7 days. The 412 KeV gamma emission of the labelled gold specimens was then counted and the average amount of gold excreted in the urine and feces calculated. Using the urinary and fecal excretion rates, pharmacokinetic indices were calculated for the single-dose studies. For each 24 hr interval, approximately twice as much gold was excreted in urine and feces of rats given the 1.0 mg as compared to the 0.5 mg Au/kg dose, but pharmacokinetic indices were similar in both groups.

The effect of chronic administration of gold on its excretion in urine and feces was also examined. A dose of 1.0 mg "cold" Au/kg was administered for 13 consecutive weeks, the 14th dose being ^{198}Au-ATM. Urine and fecal specimens were again collected at 24 ± 4 hr intervals and the amount of gold in the urine and feces, and the pharmacokinetic indices calculated as before. Urine and fecal excretion of gold after repeated administration of ATM (1.0 mg Au/kg,im) was not significantly different from excretion after a single dose of ATM (1.0 mg Au/kg,im). However, repeated administration increased the overall elimination rate constant and reduced $T_{1/2}$ Au.

To try and explain the relatively high and consistent excretion of gold in the feces, we examined the effect of the cholestatic agent alpha-naphthylisothiocyanate (ANIT), on fecal excretion. ANIT causes

reversible intrahepatic cholestasis within 24 hrs with normalization occurring within 3-6 days depending on the dose administered. Rats were gavaged with ANIT in doses of 300 and 150 mg/kg, 24 hrs before injection of ^{198}Au-ATM (1.0 mg Au/kg,im) and urine and feces collected as before. Significantly less ^{198}Au was recovered in feces within the first 72 hrs however a compensatory increase in urine gold concentration was observed. After 72 hrs, ANIT-treated rats and controls excreted similar amounts of gold in the urine and feces. Pharmacokinetic indices were similar for both ANIT-treated and control rats.

Lastly, we examined the effects of single and repeated administration of ATM on metallothionein (MT) levels in rat kidney and liver. Sprague Dawley rats were given either a single or 14 weekly injections of ATM (1.0 mg Au/kg,im). 24 hrs after the last injection, the rats were killed and the kidneys and livers removed, washed in chilled isotonic saline and either fractionated immediately using the method of Shaikh and Smith (1976) or quick-frozen (-40°C) and analyzed later. Detectable levels of a low molecular weight protein resembling MT were observed in kidney and liver cytosol of the rats examined. This material contained low concentrations of gold (detected using neutron activation analysis). No differences were found between MT levels of kidney and liver supernatants of rats receiving single or repeated injections of ATM, but gold concentrations were greater in kidney tissue.

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LIST OF ABBREVIATIONS

AAS	atomic absorption spectroscopy
ANIT	alpha-naphthylisothiocyanate
ATG	aurothioglucose
ATM	sodium aurothiomalate
ATS	sodium aurothiosulphate
BAL	British Anti-Lewisite
CCC	Cooperative Clinics Committee
CMC	carboxymethyl cellulose
cpm	counts per minute
ERC	Empire Rheumatism Council
ESR	erythrocyte sedimentation rate
hmw	high molecular weight
ia	intra-articular
im	intramuscular
JRA	juvenile rheumatoid arthritis
lmw	low molecular weight
MT	metallothionein
MW	molecular weight
NAA	neutron activation analysis
NMR	nuclear magnetic resonance
NSAIDs	non-steroidal anti-inflammatory drugs
po	orally
RA	rheumatoid arthritis
sc	subcutaneous

URINARY AND FECAL EXCRETION OF GOLD BY RATS GIVEN SODIUM AUROTHIOMALATE

INTRODUCTION

This thesis reports the investigations of the excretion kinetics of gold and the involvement of a low molecular weight metal-binding protein (metallothionein) in gold excretion.

The introduction will review the following topics:

A. Chrysotherapy in the treatment of rheumatoid arthritis
B. Toxicity from gold therapy and its treatment, and
C. Gold pharmacodynamics.

A. CHRYSOTHERAPY IN THE TREATMENT OF RHEUMATOID ARTHRITIS (RA)

Gold has been used in medicine for many centuries. At one time or another it was considered a "cure-all" for every known disease. In the thirteenth century, Roger Bacon recommended gold compounds for the treatment of leprosy (Dyson, 1929), and in 1500 Paracelsus stated that a mixture of gold and mercury was an "elixir of life" and could be used to treat syphilis and tuberculosis (Slot & Deville, 1934). No scientific evidence has been found to substantiate any of these claims.

No scientific basis was provided for the use of gold in specific medical disorders until 1890. At this time, Robert Koch demonstrated that gold cyanide inhibited the growth of tubercle bacilli *in vitro*, but was less effective when administered to animals infected with tuberculosis. This discovery led to the development of gold compounds which were used in experimental tuberculosis and later in the treatment of humans.

In 1927 Landé first reported the use of aurothioglucose (Solganol®) in the treatment of diseases other than tuberculosis. Believing that gold had a nonspecific antiseptic effect, he treated 14 patients who had poorly defined diseases of a "rheumatic" origin with aurothioglucose. He observed improvement in the patients' general appearance and significant relief from the joint pain that accompanied the disorders. A year later Forestier treated patients suffering from rheumatoid arthritis (RA) with gold thiopropanol sodium sulfonate (Allochrysine®). The treatment was based mistakenly upon the belief that RA resulted from an infection similar to tuberculosis. Because gold was effective in the treatment of tuberculosis, he thought that it would help in the management of RA (Forestier, 1932).

Forestier began treatment with 50 to 100 mg of Allochrysine® im injected weekly until a total of 1.5 to 2.0 g of gold compound had been administered. After a rest period of 6 to 8 weeks, a second similar series of injections was administered provided there were no serious toxic reactions. Gold therapy was continued in this manner for 1 to 2 years. In 70% of his 48 patients, marked reduction in joint inflammation and pain was observed. By 1935 Forestier had treated over 550 patients suffering from RA with a variety of gold compounds including Allochrysine®, sodium aurothiomalate (Myochrysine®), aurothioglucose (Solganol®) and calcium aurothioglycolate (Myoral®). In 70-80% of patients, gold effectively reduced joint inflammation and pain.

Numerous European investigators (Pemberton, 1935; Crosby, 1936; Copeman & Tegner, 1937) confirmed Forestier's observations — generally in patients refractory to other forms of treatment. The clinical trial by Hartfall, Garland & Goldie (1937a) was particularly impressive. Eighty percent of 690 patients with RA were "cured", or greatly improved, following chrysotherapy however treatment resulted in a higher incidence of toxicity (42%) and death (1%) than had been observed by Forestier.

In the United States at this time, chrysotherapy was viewed with extreme scepticism and was not considered an initial treatment for RA because of the high incidence of serious, often fatal, toxic reactions. It was customary to begin treatment with more conservative measures (Cecil, 1934; Holbrook & Hill, 1936) which included rest (both emotional and physical), a high caloric high vitamin diet, blood transfusions for existing anemia, analgesics, physical therapy, and orthopedic procedures for the prevention or correction of any deformities. Therapy with vaccines and removal of the foci of infections were popular, but no studies provided evidence of the benefit to be derived from these treatments. Only patients with unremitting progressive disease and those refractory to the conventional forms of treatment were candidates for gold therapy (Sashin, Spanbock & Kling, 1939; Snyder, Traeger & Kelly, 1939; Key, Rosenfeld & Tjoflat, 1939).

By 1940 in both Europe and the United States, gold was recognized as one of the most valuable agents for the treatment of RA. A controlled clinical study by Ellman, Lawrence & Thorold (1940) provided definitive evidence of the beneficial effects of gold on the course of the disease. RA was considered inactive in 74% of 60 patients treated for 9 months with Solganol®. By contrast, the disease was considered inactive in only one of 30 control patients who had not received gold injections. Gold ameliorated the course of the disease, not only in the advanced severe forms but also in the early cases of arthritis which were particularly responsive to gold treatment (Tarsy, 1940; Cecil, Kammerer & DePrume, 1942). However, the high incidence of toxicity associated with gold in the doses recommended (100 mg weekly) still severely limited its use.

Patch and intradermal tests were not helpful in predicting which patients were likely to develop toxicity (Key, Rosenfeld & Tjoflat, 1939). High doses of calcium gluconate, and the vitamins C and D (administered simultaneously with the gold salts) were ineffective in preventing toxicity (Tarsy, 1940), and specific antidotes for gold toxicity were not yet available.

To try and reduce the incidence of toxicity, the weekly dose and total dose of gold were reduced (Freyberg, 1942; Price & Leichtentritt, 1943; Hartung, 1943). This reduced the incidence of toxicity and did not affect the therapeutic results. Reduction of dosage, more careful monitoring of patients for early symptoms of toxicity, and the introduction of cortisone (Hench, Kendall _et al._, 1949) and dimercaprol (British Anti-Lewisite, BAL) (Cohen, Goldman & Dubbs, 1947) as antidotes, greatly reduced the frequency of serious toxic reactions.

By the late forties, most investigators had realized the importance of carefully controlled studies and began comparing the effects of chrysotherapy with placebo (Fraser, 1945; Waine, Baker & Mettier, 1947; Adams & Cecil, 1950; Snorrason, 1952; Lockie, Norcross & Riordan, 1958). Favorable results following gold treatment were reported in most studies, but some investigators found no difference between the gold-treated and control groups (Merliss, Axelrod _et al._, 1951; Brown & Currie, 1953).

Because of the conflicting reports, the Empire Rheumatism Council (ERC), in 1960, conducted a multicentre double-blind clinical trial in an attempt to determine whether gold was effective in RA. In one group of 99 patients with active RA, each received a total dose of 1.0 g of Myochrysine® in 20 injections of 50 mg im, weekly. In a second group of 100 arthritic patients (the controls), each received control injections containing Myochrysine® (0.01 mg). Patients were assessed before treatment began, at 3 months, at completion of the course of treatments, and at 18 and 30 months. Significant clinical improvement by all criteria used, except radiographic analysis, was observed more frequently among the gold-treated patients at 3, 6 and 18 months but little difference existed between the two groups at 30 months (ERC, 1961a).

Although earlier reports stressed the importance of continuing gold therapy (Forestier, 1935; Browning, Rice et al., 1947; Adams & Cecil, 1950; Freyberg, 1950; Lockie, Norcross & Riordan, 1958), the ERC did not continue gold maintenance therapy after the initial remissions. This could be the reason for the high number of relapses in the gold-treated patients at month 30.

In 1973, the Cooperative Clinics Committee (CCC) of the American Rheumatism Association conducted a similar double-blind controlled study of chrysotherapy. It differed from the ERC study in that phase II of the trial evaluated the benefit of continued therapy in prolonging remissions obtained with the initial course of gold. Half of the patients continued on gold therapy for an additional 18 months and the controls remained on placebo therapy. This maintenance program consisted of intramuscular injections of 50 mg of Myochrysine® every other week for 6 weeks followed by 6 more injections every third week and lastly, monthly injections for the rest of the 2 year study. Although the number of patients completing the trial was too small to allow definite conclusions, the results suggested that maintenance therapy was beneficial. The group in which therapy was continued showed no increase in the number of joints involved, had an improved grip strength and a fall in the erythrocyte sedimentation rate (ESR), whereas the placebo group showed deterioration in all 3 parameters. These results were confirmed in a 2-year double-blind study conducted by Sigler and co-workers (1974). Although only 27 arthritic patients were involved (13 received Myochrysine® and the others placebo), the study demonstrated that long term gold treatment had altered the natural course of the disease favorably, and, for the first time, radiographs showed that chrysotherapy had slowed or arrested the progression of the disease. More recently, Luukkainen and associates (1977 a,b) reported significantly less joint destruction in gold-treated patients than in controls who received less than 50 mg of Myochrysine® and had terminated chrysotherapy because of side effects. Patients whose RA had been active for less than 10 months initially showed greater improvement than patients who had had the disease for a longer time before commencing chrysotherapy. However, by the end of the follow-up period (68 months), all 3 gold-treated groups had significantly less radiographically demonstratable joint damage than the controls. Since then more reports have been published which confirm the value of gold for the treatment of RA (Schorn & Anderson, 1975; Majoos, Klemp et al., 1981).

Table I summarizes the results of studies published between 1935 and 1958. Freyberg (1966) provides a more complete review of the literature. The benefits of chrysotherapy were evident in the majority of arthritic patients treated with various gold compounds. The incidence of gold toxicity was approximately 30%. This cumulative clinical

Table I. Literature Review of Some Gold Studies Published Between 1935 and 1958.

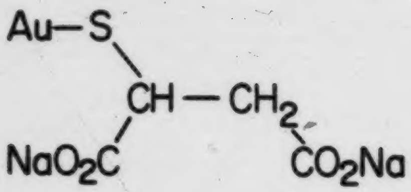
Investigator	Year	Number of Patients	Observation Period (yr)	Arrested or Greatly Improved (%)	Moderately Improved (%)	Toxicity (%)
Forestier	1935	550	6	75	—	25
Pemberton	1935	100	5	50	38	—
Phillips	1936	20	3/4	30	—	70
Crosby	1936	27	—	85.2	—	—
Copeman *et al*	1937	51	½-4	58	18	22
Hartfall *et al*	1937	690	4	66.7	13.0	41.9
Sashin *et al*	1939	80	1-5	43.7	38.7	23.8
Snyder *et al*	1939	50	—	12	36	17
Key *et al*	1939	53	—	38	—	62.8
Tarsy	1940	22	3	54.5	—	36.3
Ellman *et al*	1940	90	3/4	74	—	27.8
Cecil *et al*	1942	245	1-5	66	20	42
Price *et al*	1943	101	1-3	60	—	38
Hartung	1943	264	—	54	—	38
Graham *et al*	1943	95	1-3	67	20	54
Fraser	1945	103	1	42	21	—
Oren	1946	150	—	84.5	—	4.6
Rose	1947	91	½-1	73	11	11
Browning *et al*	1947	47	1½-6	23	—	56
Waine *et al*	1947	120	3	20.7	36.2	50
Cohen *et al*	1948	475	—	68.3	—	21
Adams *et al*	1950	106	1½-5	70.8	17	48.6
Cecil *et al*	1951	49	1-10	68	—	25
Snorrason	1952	295	4	72	—	—
Schwartz *et al*	1954	56	3	3.6	37.5	14
Lockie *et al*	1958	369	¼-25	8	49	32.2
	TOTAL	4306		66.6%		34.6%

experience agrees well with the results of more recent controlled studies.

Sabin & Warren (1940) and Preston, Block & Freyberg (1942) demonstrated that the therapeutic effectiveness of gold preparations depended on the gold in the molecule and not on sulphur or any other constituents. Since then numerous gold(I) compounds have been used to treat RA including sodium aurothiomalate (Myochrysine®), aurothioglucose (Solganol®), gold thioglycoanilide (Lauron®) and gold sodium thiosulphate (Sanochrysine®). Recently a gold(I) phosphine compound (Auranofin®) has been developed for oral use and is currently undergoing clinical trials (Finkelstein, Walz et al., 1976; Berglof, Berglof & Walz, 1978; Weisman & Hannifin, 1979; Meyers & Klemp, 1981). The structural formulas of these compounds are shown in Figure 1. Only the first two compounds listed are commonly used in Canada and the United States. Sodium aurothiomalate (ATM) contains 45% Au and is available in aqueous solution for im injection. Aurothioglucose (ATG), a water soluble organic compound, contains 50% Au; it is available as an oily suspension for im injection.

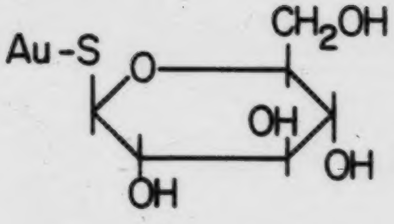
Very few controlled studies have compared the efficacy and toxicity of the various gold compounds. It is thought that ATM and ATG are equally effective in the treatment of RA (Gottlieb, 1977), but in several reports a lower incidence of toxicity has been noted with ATG (Rothermich, Philips et al., 1976; Lawrence, 1976). Certain adverse effects such as vasomotor (nitritoid) reactions (Lintz, 1941; Price & Leichtentritt, 1943; Smith, Peak et al., 1958; Austad, 1970; Sharp, Lidsky et al., 1977; Gottlieb & Brown Jr., 1977; Davison, 1980) and non-vasomotor responses (Halla, Hardin & Linn, 1977) occur most commonly with ATM. Unfortunately the use of ATG also has disadvantages.

(1)



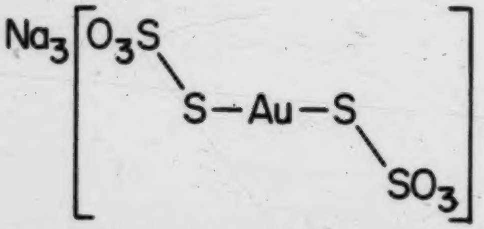
Sodium aurothiomalate
Myochrysine ® (ATM)

(2)



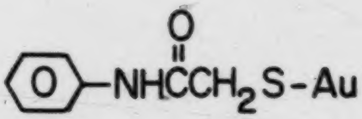
Aurothioglucose
Solganol ® (ATG)

(3)



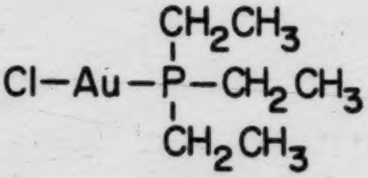
Gold sodium thiosulphate
Sanochrysine ® or
Crisalbine ® (ATS)

(4)



Gold thioglycoanilide
Lauron ®

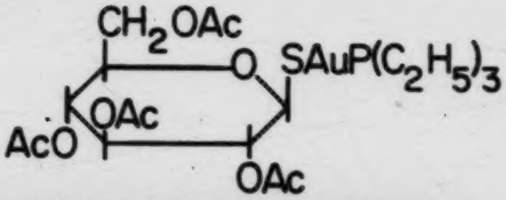
(5)



$$\mathrm{Cl-Au-\overset{CH_2CH_3}{\underset{CH_2CH_3}{P}}-CH_2CH_3}$$

Triethylphosphine gold chloride
(SK & F36914)

(6)



3 triethylphosphine gold-2,3,4,6-
tetra-O-acetyl-1-thio-β-D-
glucopyranoside. Auranofin ®

Figure 1. Structural formulas of gold compounds used in the treatment of RA. Compounds (1) and (2) are the most widely used and are administered by intramuscular injection. Compounds (3) and (4) are not used in Canada or the USA. Compound (5) is active orally as is (6) which is undergoing clinical trials.

Intramuscular injections of ATG may cause more patient discomfort than ATM because it is suspended in a viscous oil. As well, firm masses have been observed at the sites of injection possibly due to the non-absorbed oil. Also, if the vial is not well shaken just prior to an injection to resuspend the ATG particles, the dose may be incorrect.

The triethylphosphine-gold compound (Auranofin®) contains 29% gold by weight and is well absorbed from the stomach. In preliminary studies, clinical improvement was evident after the fifth week of treatment and continued until the study was completed. Arthritic patients had reduced ESRs, fewer painful and swollen joints, and increased grip strengths. Patients had significantly lower blood gold concentrations after auranofin treatment (less than 100 µg/dl) than after parenteral administration of standard doses of ATM (more than 300 µg/dl) (Finkelstein, Walz et al., 1976; Berglof, Berglof & Walz, 1978). To date, very few side effects have been observed. Some reports suggest that the gold toxicity e.g. dermatitis, stomatitis, and proteinuria occurs less frequently with auranofin than with ATM but gastrointestinal disturbances are as common (Finkelstein, Walz et al., 1976; Berglof, Berglof & Walz, 1978). Mild diarrhea and abdominal cramps have occurred early in the course of treatment disappearing on continued administration of auranofin (Gottlieb, Riskin et al., 1980). Further experience is needed with this new drug before the complete spectrum of reactions is known.

Gold is indicated for active, progressive, erosive RA that can not be controlled adequately by more conservative measures such as non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, physical therapy and rest. Early treatment is important since gold only suppresses the inflammatory process and cannot repair previous damage to cartilage and bone, nor can it restore deformed and ankylosed joints. It should not be used in patients whose arthritis is no longer active, i.e. "burned out" cases of arthritis (Gottlieb, 1977).

Chrysotherapy has been shown to be effective in the management of juvenile rheumatoid arthritis (JRA) (Hicks, Hanson & Kornreich, 1970; Ansell, 1972; Levinson, Balz & Bondi, 1977; Brewer Jr., Giannini & Barkley, 1980). The dose is usually 1.0 mg/kg body weight administered intramuscularly once a week with a maximum dose of 50 mg weekly for larger children. Side effects appear to occur as frequently in children as in adults and affect similar organs, even though lower doses are employed.

Gold by itself is not the complete treatment for RA. It should be part of a well planned program of therapy carefully adjusted to the patient's needs. For progressive RA some physicians prefer to begin treatment with penicillamine, but gold is generally considered before other slow acting, remittive anti-rheumatic drugs such as chloroquine, azathioprine, methotrexate and cyclophosphamide. These drugs are effective in the treatment of RA and also are associated with a significant number of complications (Currey, Harris et al., 1974; Huskisson, Gibson et al., 1974; Dwosh, Stein et al., 1977; Husain & Runge, 1980).

Gold should not be administered to patients with a past history of severe gold toxicity such as exfoliative dermatitis, blood dyscrasias or persistent heavy proteinuria. Previous liver and kidney diseases are not absolute contraindications unless the functions of these organs are seriously impaired. Patients with existing abnormal liver and renal function should be carefully monitored during treatment for a progression or increase in these abnormalities indicative of gold toxicity. When other serious systemic diseases exist such as diabetes, hypertension, etc., they should be controlled prior to chrysotherapy. Patients with severe allergic diseases or those known to have an idiosyncracy to other drugs should be given gold with extreme caution. Chronic dermatitis is not a contraindication to gold therapy but may complicate the early recognition of gold rashes or could be aggravated by gold. Recently some skin disorders such as pemphigus have been successfully treated with gold salts (Penneys, Eaglstein & Frost, 1976). Traditionally chrysotherapy was not employed in pregnant or lactating women because the effect on the fetus or newborn infant was not known. Reports in the literature describing the outcome of pregnancies exposed to gold in utero are few. Hollander (1972) discussed a series of uneventful pregnancies in women receiving chrysotherapy but no long-term follow-up of the offspring has been reported. More recently Rogers, et al., (1980) reported the birth of an abnormal child resulting from a pregnancy inadvertently exposed to gold throughout the first and early part of the second trimester. The visible malformations included a cleft lip and palate, a flattened nose, crumpled ears and a short neck. The infant died on the fifth day. Other causes for these malformations have not been ruled out but a possible teratogenic effect of gold must be considered.

Age is not a contraindication to the use of gold since good results have been obtained in elderly patients with well established disease (Cecil & Kammerer, 1951) as well as in patients with juvenile rheumatoid arthritis (Hicks, Hanson & Kornreich, 1970; Ansell, 1972; Levinson, Balz & Bondi, 1977; Brewer Jr., Giannini & Barkley, 1980).

Administration of gold with other slow acting remittive drugs (phenylbutazone, antimalarials, cytotoxic agents) is not recommended. Since each drug alone may cause dermatitis, bone marrow depression or proteinuria, potentiation of these effects may occur when these drugs are used together. Potential drug interactions and increased toxicity have not been studied, but must be considered when therapy with more than one agent is contemplated (Gottlieb, 1981). The use of penicillamine (a heavy metal chelating agent) along with gold is not advised. Animal studies (Eyring & Engleman, 1963; Davidson & Engleman, 1964; Rubin, Sliwinski et al., 1967) and some human studies (Eyring & Engleman, 1963; Davis, 1969) demonstrated that penicillamine significantly increased the excretion of gold when administered in doses larger than 1.0 g daily for at least 3-7 days. More recent work (Davis & Barraclough, 1977; Biggs, Boland et al., 1979), however, does not confirm the results obtained in earlier studies and the effectiveness of D-penicillamine as a chelating agent for gold toxicity is questionable. At one time concurrent use of systemic corticosteroids and gold was believed to reduce the efficacy of the chrysotherapy, however no evidence to substantiate this has been reported (Gerber & Paulus, 1975). In fact, gold salts have been used to help reduce the corticosteroid dosage in patients who have developed side effects (Ansell, 1972).

Current gold dosage schedules were derived empirically from the clinical observations of patients over many years. A typical schedule consists of an initial test dose of 10 mg of ATM or ATG followed in a week by 25 mg. Provided that no adverse effects are observed, 50 mg is then administered im weekly until a total dose of 1.0 g of gold compound (500 mg of elemental gold) has been given or toxicity occurs.

If patients go into remission before completing the full 20 week course, gold is administered at progressively longer intervals, e.g. bi-weekly for a given period then tri-weekly and then monthly to reduce the chances of toxicity developing. Most patients require a minimum of two to three months of treatment (300—500 mg of gold compound) before the therapeutic effect is observed. If, and when, toxicity develops, treatment is temporarily or permanently discontinued depending upon the type and severity of the reaction. If improvement occurs after a cumulative dose of 0.5 g Au most rheumatologists recommend maintenance therapy in order to reduce the incidence of relapses. Typically, maintenance therapy consists of injections of 50 mg bi-weekly for a period of 2-20 weeks, then tri-weekly for several months and then monthly unless a "flare-up" occurs. Rothermich et al., (1976) claimed that injections at 4 week intervals or longer were insufficient to maintain remission. More recently Griffins et al., (1981) reported that patients receiving bi-weekly injections had a much higher incidence of toxic side effects (48%) than patients on monthly injections (22%). These results suggest that monthly maintenance therapy is as effective as bi-weekly and is less likely to cause toxicity. Srinivason and co-workers (1979) clearly demonstrated the value of maintenance therapy. They reported continued remissions in 73% of RA patients who received chrysotherapy for 3 years or longer compared to only 6% "spontaneous" remissions when gold therapy was stopped during the first 18 months. Unfortunately there is a tendency for some patients to deteriorate after prolonged periods of remission despite continued chrysotherapy (Rothermich, Philips et al., 1979). For these patients, administration of another remittive drug (penicillamine, methotrexate, azathioprine or cyclophosphamide) is an alternative. If the maintenance dosage is inadequate, the disease will gradually reappear. Control can often be re-achieved by returning to weekly injections. If remission occurs, 50 mg doses should be administered bi-weekly, or larger doses (e.g. 75 mg) given tri-weekly (Smith, 1963; Sagransky & Greenwald, 1980).

After 0.5 g Au if a patient has not shown positive response, several alternatives are available. These include increasing the dosage of gold for a short time, continuing with the conventional gold dosage for a further period, or discontinuing chrysotherapy altogether. Smith et al., (1958) reported that 82% of patients unresponsive to weekly gold injections improved significantly when the dose was gradually increased at weekly intervals by 10 to 25 mg, until a maximum of 100 mg was reached. These patients were considered "hyper-excretors" of gold, and Smith recommended individualizing the dosage according to urinary excretion (1963). Unfortunately monitoring the urinary gold concentrations for all RA patients is not practical. Rothermich, et al., (1976) reported that 43% of patients who failed to respond to a conventional dose of gold improved when 1.5 to 2 times this dose was administered. Despite these larger than normal doses, side effects were observed in only 10% of the patients. Rather than gradually increasing the dosage, some physicians administer an additional 10-20 injections of 50 mg of gold (provided there are no toxic reactions) in the hope that a higher cumulative dose will be beneficial. Others discontinue chrysotherapy after administration of 0.5 g Au and begin treatment with another remittive agent.

Recently in several controlled studies, the effectiveness of the "conventional" schedule was compared to both higher and lower dosages.

Both Cats (1976), and Furst et al., (1977), demonstrated increased toxicity and no additional clinical benefit from 100 mg and 150 mg Au administered weekly. In another study, 25 mg ATM weekly was reported to be as effective as twice that amount administered on a flexible dosing schedule; this resulted in a reduction in total dosage but did not diminish the incidence of side effects (Sharp, Lidsky et al., 1977). Lorber and associates (1973) recommended individualizing the dosage of gold to maintain the serum gold concentrations above 300 μg/dl, because this concentration appeared to correlate well with a favorable clinical response. Because numerous other investigators have not been able to confirm any relationship of serum gold levels to either clinical response or toxicity, (Gerber, Paulus et al., 1972; Mascarenhas, Granda & Freyberg, 1972; Rubinstein & Dietz, 1973; Gottlieb, Smith & Smith, 1974; Furst, Levine et al., 1977), 50 mg of gold compound weekly has remained the accepted standard and is likely to remain so until more persuasive data is presented.

B. TOXICITY FROM GOLD THERAPY AND ITS TREATMENT

Controlled studies have demonstrated that chrysotherapy is of benefit to patients with progressive RA (see ERC, 1960; CCC, 1973; Sigler, Bluhm, et al., 1974). Remissions or marked improvement can be expected in 60-70% of patients, but side effects develop in about one third of these patients, resulting in discontinuation of treatment (Gibbons, 1979).

Gold causes many side effects (Table II). In most early studies, any adverse reaction or "disturbance" was attributed to gold toxicity

Table II. Adverse Reactions Associated with Chrysotherapy

Mucocutaneous

- Pruritus
- Dermatitis
- Chrysiasis
- Stomatitis
- Vaginitis
- Ocular lesions

Renal

- Proteinuria
- Nephrotic syndrome

Hematologic

- Eosinophilia
- Thrombocytopenia
- Leukopenia
- Agranulocytosis
- Aplastic anemia

Miscellaneous

- Vasomotor (nitritoid) reactions
- Non-vasomotor reactions
- Diffuse pulmonary infiltrates
- GIT disturbances
- Enterocolitis
- Cholestatic jaundice
- Peripheral neuropathy

(Hartfall, Garland & Goldie, 1937b). The development of better diagnostic techniques, different classifications of the rheumatic diseases, and the recognition of similar side effects in placebo-treated patients, should have lowered the over-all incidence of toxicity. However, the incidence has not changed since the thirties when Forestier first popularized chrysotherapy. Freyberg (1966) reported an average incidence of 32% in over 7000 patients treated with gold salts between 1935 and 1958 which is comparable to the incidence observed in more recent studies (ERC, 1961b; Cats, 1976; Majoos, Klemp et al., 1980).

Most complications of chrysotherapy are mild, but approximately 5% of RA patients treated with gold will experience severe, life-threatening reactions such as exfoliative dermatitis, nephrotic syndrome or aplastic anemia (Freyberg, 1966). The mortality rate in 1935 was reported to be as high as 3% (Hartfall, Garland & Goldie, 1937b), but is now estimated at about 0.4% (Gottlieb, 1981). This reduction in death rate can be attributed to the administration of lower doses of gold, limiting therapy to injections of ATM and ATG intramuscularly, earlier recognition of toxicity and improved treatment of the complications with corticosteroids, chelating agents, and advanced life support systems (Gottlieb, 1977).

Gold toxicity may appear at any time during treatment but is most common after the administration of 300-500 mg of Au (Jessop, 1972a; Smith, Peak et al., 1958). Patients who experience only minor side effects during the initial treatment with gold rarely experience toxicity during maintenance therapy (Cats, 1976; Kean & Anastassiades, 1979), or, when the dose of gold is increased to 50 mg weekly because of a flare-up (Sagransky & Greenwald, 1980).

Several early reports claimed a positive correlation between gold toxicity and therapeutic effect (Copeman & Tegner, 1937; Bayles & Fremont-Smith, 1956; Smith, Peak et al., 1958) but later controlled trials failed to confirm these observations (ERC, 1961a; Gottlieb, Smith & Smith, 1972; Cats, 1976). It is now generally agreed that toxicity is not a prerequisite for a favorable clinical response, and that gold toxicity may develop in the absence of any improvement (Gottlieb, 1979a).

No combination of laboratory tests and clinical examinations has been successful in predicting the development of gold toxicity. Results from patch and intradermal tests (Kay, Rosenfeld & Tjoflat, 1939; Walzer, Feinstein et al., 1972; Davis, Ezeoke et al., 1973; Penneys, Ackerman & Gottlieb, 1973), serum gold levels (Gerber, Paulus et al., 1972; Mascarenhas, Granda & Freyberg, 1972; Rubinstein & Dietz, 1973; Gottlieb, Smith & Smith, 1974; Furst, Levine et al., 1977), urinary excretion rates (Gottlieb, Smith & Smith, 1972), and gold tissue levels (Gottlieb, Smith et al., 1974b) can not be correlated with toxicity. Eosinophilia has been observed with equal frequency in patients with and without toxicity (Jessop, Dippy et al., 1974; Davis & Hughes, 1974), and in patients with active systemic RA who have not even received gold injections (Panush, Franco & Schur, 1971; Winchester, Litwin et al., 1971). Elevated serum IgM levels (Jalava, Luukkainen et al., 1977) and certain tissue antigens (HLA B12, DRW2, DRW3, DRW4) (Abruzzo, 1978; Panayi, Wooley & Batchelor, 1978; Majoos, Klemp et al., 1981) have been associated with the development of side effects in patients, however further investigation is required to establish the clinical relevance of these findings.

Attempts to prevent the development of gold toxicity by administration of large doses of vitamins, liver extracts, calcium salts and glucose have also proved unrewarding (Hartfall, Garland & Goldie, 1937b; Tarsy, 1940). It therefore seems that the only useful criteria are careful clinical and laboratory examinations of patients. Thus, during chrysotherapy, patients should be carefully monitored for toxic symptoms such as pruritus, skin rashes, sore mouths, bruising, or easy bleeding. Urinalyses and blood counts should also be performed routinely. At the first sign of a reaction, depending on the type and severity, the dosage may be reduced (Burnstein, Staley & Calabro, 1979) or gold therapy temporarily discontinued (Gottlieb, 1977; Rosenbaum, 1979). Once the lesion(s) has cleared, gold therapy may be reinstituted beginning with low doses, or if the side effect has been severe, treatment with gold should be abandoned.

1. ORGAN SYSTEMS INVOLVED IN TOXICITY

a. MUCOCUTANEOUS

Toxic reactions to gold have been reported to involve most organs in the body but the skin and mucous membranes are affected most often. Between 15—30% of all patients given gold therapy experience mucocutaneous reactions (Klinefelter, 1975; Davis, 1979). These include nondescript skin lesions, and eruptions resembling lichen planus or pityriasis rosea, which typically are pruritic (Penneys, Ackerman & Gottlieb, 1974). Exfoliative dermatitis, a much more serious complication, occurs rarely (Zvaifler, 1979; Gottlieb, 1981). Chrysiasis, the deposition of gold particles in the skin resulting in hyper-pigmentation of light exposed areas, occurs in some patients receiving prolonged treatment (Cox & Marich, 1973; Jeffery, Biggs et al., 1975). Lesions of the oral mucosa (stomatitis, glossitis, gingivitis) are uncommon and may be preceded by a sore mouth or a metallic taste (Freyberg, 1966). These reactions may occur alone, or, occasionally in association with dermatitis (Gibbons, 1979). Only one case of vaginitis associated with chrysotherapy has been reported in the literature (Webster & Juden, 1978). Ocular lesions (conjunctivitis, iritis and corneal ulceration) are infrequent (Hartfall, Garland & Goldie 1937b; Price & Leichtentritt, 1943), but corneal chrysiasis (accumulation of gold particles in the cornea) has been reported in 45-75% of patients receiving a cumulative dose of more than 1500 mg of gold (Hashimoto, Maeda et al., 1972; Gottlieb & Major, 1978).

The mechanism(s) responsible for these types of gold toxicity are unknown. Denman and Denman (1968) and Walzer and associates (1972) suggested that certain reactions to gold (e.g. dermatitis) were immunologically mediated. Although eosinophilia and/or elevated serum IgE levels have been observed in association with gold dermatitis — suggesting a Type I hypersensitivity reaction (Jessop, Dippy, et al., 1974; Davis, Ezeoke et al., 1973), similar changes have been reported in patients with active RA who have not received gold treatment (Panush, Franco & Schur, 1971; Winchester, Litwin et al., 1971; Hunder & Gleich, 1974; Grennan & Palmer, 1979). Also, patch and intradermal skin tests have been negative in most patients with gold dermatitis, and inconclusive results have been obtained in lymphocyte transformation tests (Penneys, Ackerman & Gottlieb, 1974). Because cutaneous reactions often develop after the patient has received multiple gold injections (400—800 mg cumulative dose), a direct toxic effect on the skin has been suggested. Chrysiasis has been considered an example of a cumulative toxic effect (Cox & Marich, 1973; Stone, Claffin et al., 1973). Skin biopsies of patients with chrysiasis were found to contain high levels of gold, but these patients had had no dermatitis since developing chrysiasis (Jeffrey, Biggs et al., 1975). Other investigators reported that skin from lesions and unaffected areas in the same patient contained similar amounts of gold, and these levels were comparable to those found in the skin of patients without dermatitis (Gottlieb, Smith et al., 1974b; Ghadially, DeCoteau, et al., 1978). These findings suggest that something more than the deposition of gold is required for cutaneous lesions. The possibility that skin lesions are an idiosyncratic reaction to gold has also been suggested because many patients experience toxicity after receiving only a single small dose of gold (Saphir & Ney, 1966).

Most authorities recommend the discontinuation of gold therapy when cutaneous or mucous membrane lesions occur. These manifestations generally resolve spontaneously within 3-4 months, depending on the severity, however, a few eruptions can persist much longer (Gibbons, 1979). Antihistamines may be useful for pruritus and topical steroid preparations have been used for symptomatic relief of dermatitis, stomatitis and vaginitis, but there is no evidence that they shorten the rate of resolution of these lesions substantially (Penneys, Ackerman & Gottlieb, 1974). More serious complications, such as exfoliative dermatitis, require the discontinuation of chrysotherapy and administration of systemic corticosteroids. Dimercaprol and penicillamine have been used successfully in the past (Ragan & Boots, 1947; Montgomery, 1950) but are only recommended for severe reactions unresponsive to corticosteroid therapy (Davis, 1969). Chrysiasis appears to be a long-lasting pigmentation (Jeffery, Biggs et al., 1975) of cosmetic importance only. Corneal chrysiasis does not cause visual disturbances or other ocular symptoms and does not require discontinuation of treatment. Unlike skin chrysiasis, the deposits of gold in the cornea have been reported to disappear within 3—5 months after termination of gold treatment (Hashimoto, Maeda et al., 1972). Even if the occurrence of mucocutaneous reactions leads to the discontinuation of chrysotherapy, many patients will tolerate further therapy after their lesions clear. Chrysotherapy was reinstituted successfully in 28 of 30 patients who had previously developed rash, stomatitis, or both (Kleinfelter, 1975). In some of these patients mucocutaneous reactions recurred but did not require stopping therapy. Further gold treatment for patients who have experienced severe cutaneous reactions i.e. exfoliative dermatitis is contraindicated (Penneys, Ackerman & Gottlieb, 1974).

b. RENAL

The association of renal lesions with the administration of gold in animals (Ganote, Beaver & Moses, 1966; Stuve & Galle, 1970; Nagi, Alexander & Barabas, 1971; Thompson, Blaszak et al., 1978) and in RA patients (Brun, Olsen et al., 1964; Lee, Dushkin et al., 1965; Silverberg, Kidd et al., 1970) is well documented. The most common complication is proteinuria; the incidence ranging between 0 and 26% (Table III). Proteinuria has also been observed in about 3% of placebo-treated patients (ERC, 1960; CCC, 1973). Possible causes

Table III. Incidence of Proteinuria During Chrysotherapy

Investigator	Year	Conventional Gold Dose Number of Patients Toxic/Treated	 Percent Toxic	No Gold Number of Patients Toxic/Treated	 Percent Toxic
ERC	1960	4/99	4	3/100	3
Silverberg _et_ _al._	1970	5/75	7	--	-
CCC	1973	0/36	0	1/32	3
Cats	1976	4/119	3	--	-
Rothermich _et_ _al._	1976	2/55	4	--	-
Sharp _et_ _al._	1977	6/38	16	--	-
Furst _et_ _al._	1977	6/23	26	--	-

include contaminated specimens, urinary tract infections (Davis, 1979), or other drugs used to treat arthritis i.e. analgesic nephropathy (Lancet, 1966). Nephrotic syndrome, a more serious renal complication, is much less common, reported in only 0.2-2.6% of arthritic patients on chrysotherapy (Silverberg, Kidd, _et_ _al._, 1970; Vaamonde & Hunt, 1970; Tornroth & Skrifvars, 1974). Besides gold, other causes for nephrotic syndrome include amyloidosis (Nagy El Mahallawy & Sabour, 1959; Allander, Bucht _et_ _al._, 1963) and lupus nephritis (Pollack, Pirani, _et_ _al._, 1962; Schreiner & Maher, 1965); therefore careful diagnosis is important. The mechanism(s) responsible for the production of renal lesions is not known. Gold deposits have been detected in renal tubules, interstitial tissues and glomerular tufts in kidney biopsies obtained in patients with gold-induced proteinuria or nephrotic syndrome (Silverberg, Kidd _et_ _al._, 1970; Viol, Minielly, _et_ _al._, 1977). Immunoglobulins (IgG, IgM), and complement deposits containing electron-dense gold particles were also observed within the glomeruli (Katz & Little, 1973; Yarom, Stein, _et_ _al._, 1975; Skrifvars, Tornroth & Tallquist, 1977) supporting the hypothesis that immune complexes are involved in the pathogenesis of gold-induced nephropathy. Another hypothesis which warrants further investigation is that RA itself can induce membranous nephritis (Samuels, Lee _et_ _al._, 1977).

Since most patients are asymptomatic, regular urinalyses are required to detect gold-related proteinuria and nephrotic syndrome (Davis, 1979). A trace amount of protein in the urine is not an indication to discontinue gold treatment but persistent proteinuria, in the absence of a urinary tract infection or other cause, may be an early sign of gold nephropathy, and injections should be stopped

(Jessop 1972; Gibbons, 1979). Proteinuria and nephrotic syndrome may not require treatment other than the discontinuation of chrysotherapy, but in more severe cases corticosteroids should be administered (Vaamonde & Hunt, 1970; Davis, 1979). There is no evidence that dimercaprol or penicillamine are helpful, but in cases unresponsive to corticosteroids these agents may be tried (Gibbons, 1979). Gold therapy may be reinstituted once the proteinuria has resolved but is not given again to patients who have experienced more serious renal side effects.

c. HEMATOLOGIC

Hematologic disorders resulting from chrysotherapy include eosinophilia, thrombocytopenia, leukopenia or agranulocytosis and aplastic anemia. The most common hematological change associated with gold is eosinophilia which has been reported in 40—45% of gold-treated patients (Davis & Hughes, 1974; Jessop, Dippy, et al., 1974). Thrombocytopenia is a less common complication of gold treatment occurring in 1-3% of patients (Gottlieb, 1981) and leukopenia may develop during treatment owing to granulocytopenia or agranulocytosis. The exact incidence is not known but is probably < 1% (Gibbons, 1979). The most serious complication is aplastic anemia. It is rare, but is associated with a 60% mortality rate (McCarty, Brill & Harrop, 1962; Kay, 1976).

An immunologic mechanism has been suggested as the cause of some of the gold-related hematological side effects. Bone marrow biopsies from patients with gold-induced thrombocytopenia have been found to contain normal or increased numbers of megakaryocytes. These data suggest that thrombocytopenia is most likely due to peripheral destruction of platelets (Stavem, Stromme & Bull, 1968; Deren, Masi, et al., 1974) even though anti-platelet antibodies have not been demonstrated (Levin, McMillan, et al., 1975). A direct toxic effect on the bone marrow has also been suggested (Howell, Gumpel & Watts, 1975; Kurnick, Robinson & Dickey, 1971).

The clinical significance of eosinophilia and leukopenia is not known. Both conditions have been reported to occur in the absence of chrysotherapy (Panush, Franco & Schur, 1971; Winchester, Litwin, et al., 1971; Barnes, Turnbull & Vernon-Roberts, 1971) and arthritic patients with pre-existing leukopenia have been successfully treated with gold salts without adverse hematologic effects (Gowans & Salami, 1973). Eosinophilia and leukopenia can be considered indications to discontinue chrysotherapy temporarily (Gottlieb, 1977), but gold can be continued in full or reduced dosage unless the situation worsens. Thrombocytopenia, an unpredictable potentially life-threatening complication, usually requires immediate termination of therapy and administration of steroids, but it can resolve spontaneously (Kay, 1976; Davis, 1979). For cases unresponsive to steroids, splenectomy (Watson, 1953; McCarty, Brill & Harrop, 1962), dimercaprol (Lockie, Norcrosse & George, 1947; Thompson, Sinclair & Duthie, 1954; Hazlett & Yendt, 1958; Saphir & Ney, 1966; Stavem, Stromme & Bull, 1968; England & Smith, 1972; Stafford & Crosby, 1978), D-penicillamine (Bluhm, Sigler, et al., 1962; Harth, Hickey et al., 1978) and vincristine (Ahn, Harrington, et al., 1974; Ball, 1977) have been used with some success. Marrow aplasia is also unpredictable and a progressive drop in hematologic values should result in cessation of chrysotherapy and immediate investigation of the bone marrow (Baldwin, Storb, et al., 1977; Davis 1979). There are no specific measures for the treatment of agranulocytosis and bone marrow aplasia but patients may benefit from corticosteroids, androgens and transfusions. Dimercaprol and D-penicillamine have been used as chelating agents although there is little evidence that they are of benefit (Kay, 1976). Generally, gold-induced aplastic anemia has a poor prognosis even with these treatments. More recently, peritoneal dialysis (Combs, Dentino, et al., 1976) and bone marrow transplantation (Baldwin, Storb, et al., 1977) have been used. For patients who have experienced hematologic complications, further gold therapy is contra-indicated.

d. OTHER GOLD-INDUCED SIDE EFFECTS

Vasomotor (nitritoid) reactions have been observed in 34% of RA patients treated with ATM (Furst, Levine, et al., 1977) however in most studies the incidence of this reaction has not been determined. Symptoms usually occur shortly after the administration of ATM and consist of facial flushing, sweating, weakness, and giddiness (Austad, 1970; Sharp, Lidsky, et al., 1977). The etiology of this reaction is not known but may involve hypersensitivity to the compound, the preservative, or the vehicle. The reaction is usually mild and passes in a short time but in some patients it is severe enough to warrant a reduction in dosage or a change to another gold compound. Subsequent reactions occur rarely. Gottlieb and Brown Jr. (1977) and Harris (1977) each reported a single case of an acute myocardial infarction as a consequence of a gold-induced vasomotor reaction.

Non-vasomotor (postinjection) reactions consisting of increased stiffness, arthralgias, myalgias, joint pain and constitutional symptoms, developed in 15% of patients treated with ATM in one study

(Halla, Hardin & Linn, 1977). The cause(s) is/are not known but the results from one study suggested that these side effects were the result of a transient inhibitory effect of ATM on the reticuloendothelial system (Williams, Lockwood & Pussell, 1979). These manifestations are well tolerated usually, but in patients who find the symptoms distressful, often ATG can be substituted without further reactions developing.

Other organ systems infrequently affected by gold include the lungs, the gastrointestinal tract, the liver and the peripheral nervous system. Acute respiratory distress associated with diffuse pulmonary infiltration has only recently been described (Winterbauer, Wilske & Wheels, 1976; Geddes & Brostoff, 1976; Gould, McCormack & Palmer, 1977; James, Whimster & Hamilton, 1978; Smith & Ball, 1980; Podell, Klinenberg, et al., 1980). Gastrointestinal side effects which have been observed frequently during gold therapy include anorexia, nausea, diarrhea and abdominal cramps (Freyberg, 1966) but only a few cases of enterocolitis have been reported in the literature (Kaplinsky, Pras & Frankl, 1973; Stein & Urowitz, 1976). Cholestatic jaundice associated with chrysotherapy is also uncommon (Favreau, Tannenbaum & Lough, 1977) as is involvement of the peripheral nervous system (VanSlype & Burniat, 1964; Walsh, 1970; Katrak, Pollack, et al., 1980). The etiology of these rare manifestations of gold toxicity has not been determined. Discontinuation of chrysotherapy and administration of systemic corticosteroids is usually effective in the treatment of pulmonary infiltrates and enterocolitis. Jaundice and peripheral neuropathies usually resolve slowly after gold treatment has been stopped. Chrysotherapy is not reinstituted in patients who have developed these side effects.

C. GOLD PHARMACODYNAMICS

1. ABSORPTION

The pharmacodynamics of gold are related at least in part to the physical properties of the carrier molecule (e.g. water solubility, particle size). The water soluble compounds such as ATM and ATS are rapidly absorbed from the injection site after intramuscular administration (Block, Buchanan & Freyberg, 1941). ATG in an oily suspension is more slowly absorbed from the injection site but otherwise behaves similarly to ATM and ATS administered in aqueous solutions (Freyberg, Block _et al._, 1941; 1942). Calcium aurothiomalate which is less soluble than ATM and ATS, follows an intermediate pattern between the soluble and insoluble gold compounds (Block, Buchanan & Freyberg, 1942). Tissue-distribution studies in animals revealed that large amounts of gold (especially colloidal gold) remained at the injection site for as long as 85 days after the last dose was administered (Block, Buchanan & Freyberg, 1941; 1942; 1944). In RA patients given ^{198}Au-ATM im, considerable activity at the injection site was encountered for up to 20 days (Lawrence, 1961). Since gold compounds are usually poorly absorbed from the gastrointestinal tract after oral administration (Sairanen & Vahatalo, 1973) gold compounds were developed that were active orally (Sutton, McGusty _et al._, 1972). Examples of these compounds include triethylphosphine gold chloride and auranofin® (S-triethylphosphine gold 2,3,4,6-tetra-o-acetyl-1-thio-β-D-glucopyranoside) (Figure 1). Oral administration of these compounds to guinea pigs inhibited erythema as effectively as a similar dose of ATM administered intramuscularly (Kamel, Brown et al., 1978a) and in RA patients, auranofin (3 mg two or three times daily) was well-absorbed and well tolerated (Finkelstein, Walz et al., 1976). Gold was detected in the serum as early as the seventeenth day, however, significantly lower levels were observed than after parenteral gold therapy (Walz, DiMartino et al., 1972; 1976). Intra-articular administration of gold salts has received little attention although a few physicians have reported success when ATM was injected into the knee joint(s). In addition to the reduction of inflammation in the knees, some patients experienced a generalized improvement in their condition indicating that ATM was absorbed systemically (Ansell, Crook et al., 1963; Lewis & Ziff, 1966).

2. SERUM PHARMACODYNAMICS

After intramuscular administration of ATM or ATS, an immediate rapid rise in plasma (serum) gold concentration is observed. Peak levels of 400-800 µg/dl are usually reached within six hours of injection and fall gradually over the next week (Freyberg, Block & Levey, 1941; Gerber, Paulus et al., 1972a; Mascarenhas, Granda & Freyberg, 1972; Jessop & Johns, 1973; Palmer & Dunckley, 1973; Lorber, Atkins et al., 1973; Harth, 1974; Gottlieb, Smith & Smith, 1974a). The lowest concentration is observed usually on the seventh day just before the next injection (Krusius, Markkanen & Peltola, 1970; Lorber, Atkins et al., 1973; Leroy, Bureau et al., 1978). Lower plasma-gold levels are observed when ATG is administered (about one-third less than with ATM or ATS) because of the slower absorption from the oil-based injection, but at the end of one week, plasma gold levels are comparable for all three compounds (Freyberg, Block & Wells, 1942; Mascarenhas, Granda & Freyberg, 1972; Rubinstein & Dietz, 1973). With increasing weekly dosages, a stepwise increase in plasma gold concentration is observed but by the sixth to eighth injection, a plateau is reached (Freyberg, Block & Levey, 1941; Mascarenhas, Granda & Freyberg, 1972; Jessop & Johns, 1973; Palmer & Dunckley, 1973; Lorber, Atkins et al., 1973; Leroy, Bureau et al., 1978). As treatment continues with a constant dose, gold does not accumulate further in the plasma. Considerable individual variation exists and plasma gold levels are affected by the nature of the gold compound administered, the dose, the frequency of dosing, the time of sampling and patient weight (Leroy, Bureau et al., 1978). At steady state, serum gold levels have ranged from 150—600 μg/dl (Gerber, Paulus et al., 1972a; Lorber, Atkins et al., 1973; Jessop & Johns, 1973; Lorber, 1977a). When the period between injections is lengthened (i.e. maintenance therapy), there is a steady drop in plasma gold until the next injection. Serum gold concentrations remain between 75 and 125 μg/dl when 50 mg of gold (ATM) was administered every third or fourth week (Gottlieb, Smith & Smith, 1974a). When gold therapy is discontinued plasma gold levels follow a pattern similar to maintenance therapy but small amounts of gold are still detectable in the plasma months or even years later (Freyberg, Block & Levey, 1941; Rubinstein & Dietz, 1973; Vernon-Roberts, Dore et al., 1976). Blood-Au levels of 300 μg/dl were observed in one patient who had died two years after receiving her last injection of gold, eventhough she had had numerous transfusions (11.1 L of packed cells) (Kamel, Brown et al., 1976a). These findings indicate that gold from the tissues must pass extremely slowly into the bloodstream for excretion. In guinea pigs, after oral administration of triethylphosphine gold chloride (18.7 mgAu/kg), serum gold levels peaked within 4 to 5 hours but were significantly lower than those following injection of ATM (15 mgAu/kg, im). However, at 24 hours, the serum gold levels were comparable (Kamel, Brown _et_ _al._, 1978a). In RA patients on auranofin therapy, whole blood-gold concentrations rose steadily during the 12 week clinical trial, reaching a maximum of 90 μg/dl with 6 mg per day dosing and 120 μg/dl with 9 mg per day. After 7 weeks of treatment, all patients had a gold level of at least 300 μg/dl in blood, however, auranofin blood gold levels did not reach a steady state or plateau, unlike ATM (Finkelstein, Walz _et_ _al._, 1976; Berglof, Berglof & Walz, 1978). After terminating auranofin therapy, serum gold levels declined slowly (Berglof, Berglof & Walz, 1978).

Numerous studies _in_ _vitro_ and _in_ _vivo_ have shown that at gold concentrations reached during chrysotherapy, 85-95% of the gold in the circulation is bound to albumin (Sliwinski, 1968; McQueen & Dykes, 1969; Mascarenhas, Granda & Freyberg, 1972; Danpure, 1974; Mason & McQueen, 1976; Kamel, Brown _et_ _al._, 1977; Ward, Danpure & Fyfe, 1977; Schattenkirchner & Grobenski, 1977; Lawson, Danpure & Fyfe, 1977; Francois, Goldberg _et_ _al._, 1978; Van de Stadt & Abbo-Tilstra, 1980). Plasma and serum gold levels are similar indicating no appreciable binding by fibrinogen (Sliwinski, 1968; McQueen & Dykes, 1969; Mascarenhas, Granda & Freyberg, 1972; Schattenkirchner & Grobenski, 1977; Francois, Goldberg _et_ _al._, 1978; Van de Stadt & Abbo-Tilstra, 1980) contrary to the findings of Lawrence (1961). Small amounts of gold (<10%) have been reported to bind to globulins (Simon, 1954;

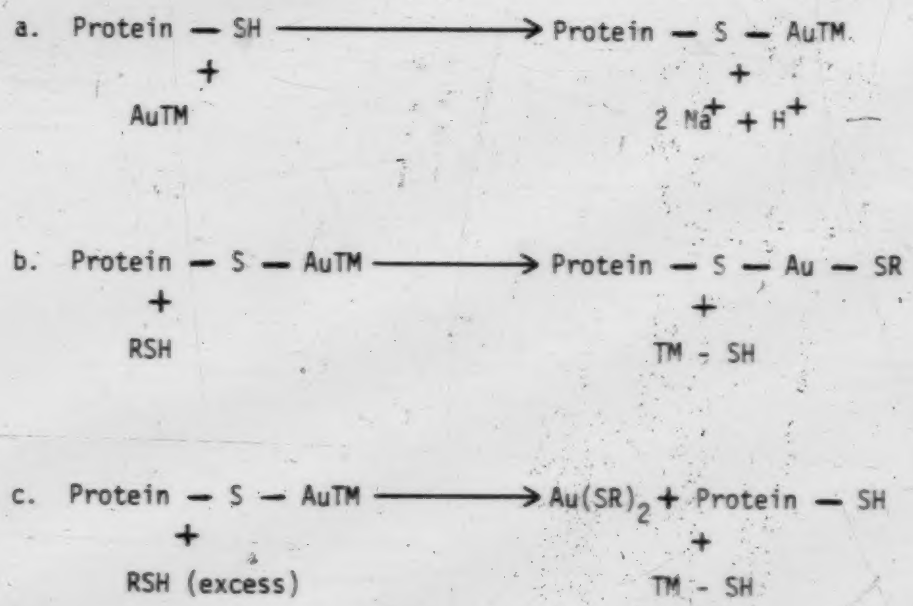
Sliwinski, 1968; McQueen & Dykes, 1969; Mascarenhas, Granda & Freyberg, 1972; Kamel, Brown et al., 1977; Lawson, Danpure & Fyfe, 1977) including α_1 globulin (Lawrence, 1961) β-lipoprotein, and 7Sγ globulin (Coke, 1963). At higher plasma gold concentrations (300-700 μg/dl), there was an increased binding of gold to immunoglobulins and complement (Lorber, Bovy & Chang, 1972; Schultz, Gottlieb & Arnold, 1973; Ward, Danpure & Fyfe, 1977; Burge, Fearon & Austen, 1978; Hasselbacher, 1981). Also, a small amount of a low molecular weight (l.m.w.) gold-containing fraction was detected in the serum (Ward, Danpure & Fyfe, 1977; Francois, Goldberg et al., 1978; Danpure, Fyfe & Gumpel, 1979). This fraction, sometimes mislabelled as "free gold" (Danpure, 1977) disappeared within 30 to 100 min. after administration (Danpure, Fyfe & Gumpel, 1979) which probably accounts for the failure of previous workers to detect it (Libenson, 1945; Kamel, Brown et al., 1976b). When ATM (30 mg/kg) was administered to guinea pigs, this l.m.w. fraction contained considerable amounts of gold 30 minutes after the injection, and it was still detectable after 24 hr. (Kamel, Brown et al., 1978a). It has been suggested that the high levels of gold observed immediately after injection are due to unchanged ATM in the serum and that cysteine and/or glutathione-gold complexes released from the tissues, are responsible for the small amounts observed in the l.m.w. fraction after 24 hr. (Danpure, 1977; Lawson, Danpure & Fyfe, 1977; Danpure, Fyfe & Gumpel, 1979).

Freyberg et al., (1941; 1942), using a colorimetric micro-chemical method found very little gold in the blood cells of patients on chrysotherapy. However, with more sensitive and sophisticated techniques such as atomic absorption spectroscopy (AAS) and neutron activation analysis

(NAA), varying amounts of gold have been detected in the cellular fractions of blood (Lawrence, 1961; Kamel, Brown et al., 1976b; Francois, Goldberg et al., 1978; Van de Stadt & Abbo-Tilstra, 1980). In two studies, 40-45% of RA patients given ATM or ATG had measurable amounts of gold in their red cell fractions (Smith, Smith & Gottlieb, 1973; Van de Stadt & Abbo-Tilstra, 1980). The amount of gold bound to erythrocytes ranged from 0-131 µg/dl of the washed red cell fraction (Smith, Smith & Gottlieb, 1973; Moller Pedersen & Moller Graabaek, 1980). ATM did not penetrate the erythrocyte membrane but appeared to be bound to it (Jellum, Munthe et al., 1979) and could not be removed by washing with free cysteine or albumin-containing media, indicating very strong binding (Kamel, Brown et al., 1976b; Van de Stadt & Abbo-Tilstra, 1980). The chrysotherapy program, the patients' blood groups and types, and results of their serologic tests, did not correlate with gold uptake into red blood cells (Van de Stadt & Abbo-Tilstra, 1980). Gold was barely detectable in red cell fractions eight hours after injection, but reached a maximum concentration by day 7 suggesting that gold is incorporated into the red cell precursors in the bone marrow and subsequently released into the circulation. This hypothesis is also supported by the findings that patients with and without red cell gold have similar serum gold concentrations (Smith, Smith & Gottlieb, 1973). In addition to gold binding by erythrocytes, Lorber and associates (1975; 1977b) have observed an increase in cell-gold content when lymphocytes were incubated with high concentrations of gold (300-700 µg/dl). Also, a fourfold increase in lymphocyte gold was observed in 10 of 17 RA patients after an im injection of ATM (Lorber, Wilcox et al., 1979). The clinical significance of these findings is not known.

After oral administration of auranofin, the distribution of gold in blood is different than after intramuscular administration of ATM. The absolute amount of gold (auranofin) bound to albumin is lower than after ATM (Walz, DiMartino et al., 1976), but the albumin, α_1, α_2 and β globulin fractions contain similar percentages of the total serum gold. The γ globulin-gold concentration was three times greater in triethylphosphine gold chloride-treated animals (Kamel, Brown et al., 1978b). Kamel et al., (1978b) reported that 25% of serum gold in the ATM, and 100% in the triethylphosphine gold-treated animals was protein bound 30 minutes after administration. However, after 24 hr., 88% of the gold (ATM) was bound to the serum proteins. Only 4% of gold as ATM has been reported to bind to the cellular fraction(s) in blood (Kamel, Brown et al., 1977) compared to 52-57% to the formed elements of the blood with auranofin (Walz, Griswold et al., 1979). ^{31}P nuclear magnetic resonance (nmr) studies demonstrated triethylphosphine gold chloride membrane-binding to the sulphydryl groups of glutathione and hemaglobin and possibly to the cell membrane(s) (Malik, Otiko & Sadler, 1980). Gold compounds which can be absorbed orally have a much higher affinity for lymphocytes than ATM for example. Four weeks after the start of auranofin therapy, lymphocyte gold content approached that obtained with ATM despite significantly lower plasma gold levels. These results suggest that as auranofin treatment continues, lymphocyte-membrane sites may eventually become saturated (Walz, Griswold et al., 1979).

Following the demonstration that gold in plasma or serum was bound mainly to albumin (McQueen & Dykes, 1969; Mascarenhas, Granda & Freyberg, 1972; Danpure, 1974) numerous investigators examined the binding parameters of this reaction. Libenson (1945) was the first to suggest that sulphydryl groups of proteins were involved in the gold binding. This was confirmed by Gerber (1964) and Danpure (1976a) who demonstrated that gold (ATM) was bound to the cysteine-34 of mercaptalbumin (Cornell & Kaplan, 1978 a,b). However, until recently, the role of carrier ligands in the gold-albumin reactions was unclear. Using nmr (Isab & Sadler, 1976), extended x-ray absorption fine structure analysis (Mazid, Razi & Sadler, 1980), and Mössbauer spectroscopy (Brown, Parish & McAuliffe, 1981), it was shown that the gold(I) atom in ATM and ATG was bonded to two bridging sulfur atoms, and that a linear 2-coordinated gold(I) structure was the most stable and therefore the most favored. When these compounds reacted with a protein such as albumin (Gerber, 1964) or a non-protein thiol such as cysteine (Danpure, 1976b) or glutathione, the bridging sulphurs were displaced to form bis-thiolato-gold(I) complexes as shown in Figure 2a (Shaw, 1979). A further reaction with non-protein thiols could lead to displacement of the thiomalate carrier ligand (Figure 2b). This was observed when ^{35}S-labelled ATM was incubated with human serum _in vitro_ and then fractionated by gel chromatography (Jellum, Aasethe & Munthe, 1977). Following the administration _in vivo_ of doubly labelled ATG, different amounts of ^{198}Au and ^{35}S-thioglucose accumulated in mouse organs, indicating dissociation of the thioglucose from gold (Swartz, Christian & Andrew, 1960). Also, in studies involving both RA patients treated with Myochrysine® (Heininger, Munthe _et al._, 1978), and mice given ^{195}Au-^{14}C-thiomalate (Jellum, Munthe _et al._, 1979; 1980), free thiomalate was detected in the urine 12 to 24 hr. after administration, but not



a. $\text{Protein}-\text{SH} + \text{AuTM} \longrightarrow \text{Protein}-\text{S}-\text{AuTM} + 2\,\text{Na}^{+} + \text{H}^{+}$

b. $\text{Protein}-\text{S}-\text{AuTM} + \text{RSH} \longrightarrow \text{Protein}-\text{S}-\text{Au}-\text{SR} + \text{TM}-\text{SH}$

c. $\text{Protein}-\text{S}-\text{AuTM} + \text{RSH (excess)} \longrightarrow \text{Au(SR)}_2 + \text{Protein}-\text{SH} + \text{TM}-\text{SH}$

Figure 2. Possible gold-protein reactions suggested by Shaw (1981).

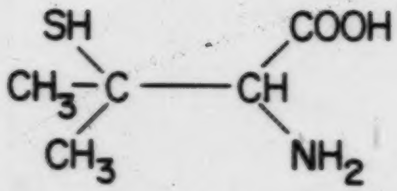
2a. Formation of a bis-thiolato-gold(I) complex

2b. Involvement of a second thiol-containing compound (RSH) with release of free thiomalate (TM-SH)

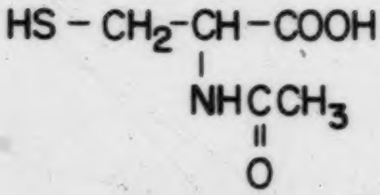
2c. Displacement of gold from protein in the presence of excess thiol (RSH) in serum or synovial fluid. Indirect evidence for thiomalate-Au splitting was reported by Griffin and Stevens (1982). In the presence of N-acetyl-cysteine or bovine serum albumin, ATM dissociated inhibiting the enzymatic activities of trypsin. Inhibition was reversed when high concentrations of imidazole was added to the system presumably because imidazole acted as a competitive binding agent for gold. The thiol-containing enzyme papain was reactivated in the presence of low concentrations of ATM due to dissociation of gold from thiomalate, but at higher concentrations the gold bound to the thiol in the active centre of the enzyme causing inhibition. Displacement of gold from the protein (Figure 2c) is another possible reaction in the presence of excess thiol (Shaw, 1981). Studies with penicillamine (Brown, McKinley & Smith, 1978; Biggs, Boland et al., 1979; Schaeffer, Shaw et al., 1980) and N-acetylcysteine (Lorber, Baumgartner et al., 1973), the structures of which are shown in Figure 3, demonstrated that substantial excesses of thiol were required to compete effectively with the protein(s) for gold. This may result from the larger binding constants for gold-protein complexes than simple thiols (Schaeffer, Shaw et al., 1980) or from sulphydryl-disulphide interchange reactions of thiols with proteins (Lorber, Chang et al., 1970).

The kinetics of gold binding to albumin have not been examined in detail. Danpure et al., (1979) demonstrated that the rate of binding of ATM to albumin in vivo was much faster (100 min.) than binding in vitro, which required approximately 5 hr. to attain equilibrium. When commercially-prepared human albumin or stored serum was used, the rate of binding to albumin was even slower. Shaw (1979) suggested that the faster reaction in vivo was due to the reaction of ATM with non-protein

(1)



(2)



$HS-CH_2-CH(NHC(=O)CH_3)-COOH$

Figure 3. Structural formulas of penicillamine (1) and N-acetylcysteine (2).

thiols to form complexes which reacted more readily with the albumin. Others (Isab & Sadler, 1976; Petering, 1976) proposed that the polymeric structure of ATM was the cause of the slow reaction with albumin. However, conformational changes in albumin e.g. the rearrangement of the disulphide bridges to an aged form (Danpure & Fyfe, 1976; Cornell & Kaplan, 1978a,b) are other possible causes for the slow reaction. Recently, several investigators (Mason, 1977a; Biggs, Boland et al., 1979; Moller Pederson, 1981) have measured the binding constants of ATM and human serum albumin (HSA). Widely divergent results were reported, and the only point of agreement was that there were at least two classes of binding sites. One class with a single binding site has a high affinity for gold. The intrinsic association constant was reported as $6.1\times10^{3}M^{-1}$ (Mason, 1977a), $1.75\times10^{6}M^{-1}$ (Biggs, Boland et al., 1979), and $3.0\times10^{4}M^{-1}$ (Moller Pederson, 1981). The second class, consisting of one or more binding sites, was reported to have a lower affinity for gold with intrinsic association constants of $2.35\times10^{2}M^{-1}$ (Mason, 1977a) or in the order of $10^{3}M^{-1}$ (Moller Pederson, 1981). The heterogeneity of serum albumin in vivo was probably not taken into account in these investigations and probably accounts for the discrepancies (Danpure 1976a; Danpure & Fyfe, 1976).

3. TISSUE DISTRIBUTION

In animals receiving ATM or other gold compounds by injection (Block, Buchanan & Freyberg, 1941; 1942; 1944; Bertrand, Waine & Tobias, 1948; Jeffrey, Freundlich & Bailey, 1958; Ganz & Brucer, 1958; Swartz, Christian & Andrew, 1960; Tonna, Brecker et al., 1963;

McQueen & Dykes, 1969; Sugawa-Katayama, Koishi & Danbara, 1975), and in RA patients receiving standard chrysotherapy (Lawrence, 1961; Silverberg, Kidd _et al._, 1970; Kantor, Harley _et al._, 1971; Gottlieb, Smith & Smith, 1972a; Grahame, Billings _et al._, 1974; Kamel, Brown _et al._, 1976a; Vernon-Roberts, Dore _et al._, 1976), tissue distribution studies have shown that the highest concentrations of gold are found in the organs containing reticuloendothelial cells such as the kidney, liver, spleen, bone marrow, adrenals and lymph nodes. Small amounts of gold also have been detected in the heart, lungs, brain tissue (Jeffrey, Freundlich & Bailey, 1958; Kamel, Brown _et al._, 1978b) muscle (Grahame, Billings _et al._, 1974), synovia (Freyberg, Block & Levey, 1941; Bertrand, Waine & Tobias, 1948; Lawrence, 1961; Gottlieb, Smith & Smith, 1972a; Gerber, Paulus _et al._, 1972a; Grahame, Billings _et al._, 1974). After prolonged chrysotherapy, skin contains considerable amounts of gold (Freyberg, Block & Wells, 1942; Bogg, 1958; Cox & Marich, 1973; Gottlieb, Smith _et al._, 1974b; Jeffrey, Biggs _et al._, 1975; Penneys, Kramer & Gottlieb, 1975). These studies all indicate that gold is widely distributed in the body. When gold was administered orally as triethylphosphine gold chloride or auranofin, different tissue distributions were observed. Stomach gold levels were very high immediately after administration of the oral gold compounds but the gold concentrations in the kidney, liver and spleen were exceptionally low, even after 7 days (Walz, DiMartino _et al._, 1972; 1976). These results suggest that the tissue concentrations are controlled by the rate of gastrointestinal absorption and not by the rate of renal elimination as in the case of the injected soluble gold compounds (Kamel, Brown _et al._, 1978b).

a. KIDNEY

The kidney contained the highest concentrations of gold as early as 48 hours after the administration of ATM (Sharma & McQueen, 1980), but maximal concentrations in subcellular organelles were found only after a considerable time (>1 month) (Mason, Sharma & McQueen, 1981). Deposits of gold were seen occasionally in the epithelial cells of the glomeruli (Brun, Olsen et al., 1964; Strunk & Ziff, 1968; 1970; Silverberg, Kidd et al., 1970), particularly during the first 2 days (Yarom, Stein et al., 1975a) indicating movement of gold through the capillary membranes. However, most gold deposits were detected in the epithelial cells of the proximal tubules (Stuve & Galle, 1970; Nagi, Alexander & Barabas, 1971; Yarom, Stein et al., 1975a,b) within hours of the administration of gold salts (Brun, Olsen et al., 1964; Ganote, Beaver & Moses, 1966) whatever the route of administration (Yarom, Stein et al., 1975a). The high concentrations of gold in the proximal tubular cells were correlated with the renal clearance of gold (Lorber, Atkins et al., 1969; Kamel, Brown et al., 1978b) supporting the suggestion that the accumulation was due to a high luminal concentration (Ganote, Beaver & Moses, 1966). Small amounts of gold were found in the epithelial cells of the distal tubules (Nagi, Alexander & Barabas, 1971; Vernon-Roberts, Dore et al., 1976) even 1 to 4 yrs. after the last injection (Brun, Olsen et al., 1964) indicating very slow mobilization of gold from the kidney. After the administration of ATM, gold was present in most of the subcellular organelles (Sharma & McQueen, 1979) and in the cytoplasm of renal cortex cells (Thompson, Blaszak et al., 1978). However, the gold in the nuclear fraction was shown later to be an artifact of the fractionation procedure, resulting from gold-containing lysosomes, "aurosomes" (Ghadially, Thompson & Lalonde, 1977), which pelleted with the nuclei because of their greater mass and density (Mogilnicka & Piotrowski, 1979; Shaw, Thompson et al., 1981). When intact rat-kidney slices were examined by energy dispersive x-ray analysis (Ghadially, 1979) and electron microscopy (Shaw, 1981), gold deposits were not observed in the nuclei. The association of gold with nuclear membranes is suggested by indirect evidence only from in vitro studies (Vernon-Roberts, Jessop & Dore, 1973; Shaw, 1981). Electron microscopy and electron probe examinations have shown that gold is present in the mitochondria of renal cells (Stuve & Galle, 1970; Nagi, Alexander & Barabas, 1971; Yarom, Stein et al., 1975b). This could be an effect of the carrier ligand on a transport system into the mitochondria as is believed is the case with sodium aurothiopropanolsulphonate, Allochrysine®, (Stuve & Galle, 1970) or it could be due to the anomalous behavior of aurosomes (Shaw, 1981). Indirect evidence suggests the interaction of gold complexes with the mitochondria in various tissues (Block & Knapp, 1945; Whitehouse, 1964; Whitehouse & Bostrom, 1965; Abou-Khalil, Yunis & Abou-Khalil, 1981). Gold deposits have also been found in the lysosomes of kidney cells (Ganote, Beaver & Moses, 1966; Strunk & Ziff, 1970; Yarom, Stein et al., 1975b) and gold may act to stabilize the membranes or inhibit various lysosomal enzymes (Persellin & Ziff, 1966; Ennis, Granda & Posner, 1968; Davies, Lloyd & Beck, 1971; Vernon-Roberts, Jessop & Dore, 1973). Recently substantial amounts of gold have been found in the cytoplasm of renal cortex cells after the administration of gold chloride (Mogilnicka & Piotrowski, 1977; 1979; Thompson, Blaszak et al., 1978), ATM (Thompson, Blaszak et al., 1978; Sharma & McQueen, 1980; Schmitz, Minkel et al., 1980), ATG (Thompson, Blaszak et al., 1978) and triethylphosphine gold chloride (Thompson, Blaszak et al., 1978). At least 4 forms of gold were resolved by fractionation using gel-permeation chromatography. Two were high molecular weight (h.m.w.) gold-binding fractions (MW > 100,000 daltons), one eluted with an apparent molecular weight of 10-12,000 daltons and resembled metallothionein (MT) and the last, was a l.m.w. non-protein species (Thompson, Blaszak et al., 1978). This was thought at first to be a gold-glutathione complex because glutathione was most abundant in the cells (Winge, Premakumar & Rajagopalan, 1975) but it has since been identified as L-cysteinato-gold(I) (Shaw, 1979). The relative amounts of the 4 forms of gold have been shown to depend on the extent of kidney accumulation and not on the differences among the gold compounds (Shaw, 1979). Also it was demonstrated that the gold in the cytosol was in labile equilibrium with protein and non-protein binding sites. At low kidney gold concentrations, e.g. with triethylphosphine gold chloride, approximately equal amounts of gold were measured in the h.m.w. and MT peaks. When higher doses were administered gold was found in the l.m.w. non-protein fraction (Shaw, Thompson et al., 1981). A similar pattern was reproduced *in vitro* by varying the concentration of ATM in the cytosol (Thompson, Blaszak et al., 1978). At very high concentrations, the h.m.w. fractions became saturated with a subsequent increase in the binding of gold to MT and the l.m.w. form. Addition of cysteine or glutathione to the cytosol caused a redistribution of gold from the h.m.w. proteins to the l.m.w. non-protein fraction without significantly altering MT-bound gold (Thompson, Blaszak et al., 1978). These findings support the concept of dynamic chemical equilibrium between gold and cytosolic proteins. Of the 4 cytosolic fractions, MT, the 10-12,000 dalton species has received the most attention. This protein which has an unusually high cysteine content (Kagi, Himmelhoch et al., 1974; Kagi, Kojima et al., 1980) can be induced in vivo in the kidney and liver of man (Pulido, Kagi & Vallee, 1966; Buhler & Kagi, 1974) and other animals (Webb, 1972; Weser, Donay & Rupp, 1973; Kagi, Kojima et al., 1980), and in cell cultures (Rugstad & Norseth, 1975; Onosaka & Cherian, 1981) after exposure to certain metals such as cadmium (Nordberg, Piscator & Lind, 1971; Webb, 1972; Winge & Rajagopalan, 1972; Weser, Donay & Rupp, 1973; Piotrowski, Trojanowska et al., 1974; Winge, Premakumar & Rajagopalan, 1974; 1978), mercury (Webb, 1972; Piotrowski, Trojanowska et al., 1974; Winge, Premakumar & Rajagopalan, 1974; 1978), silver (Winge, Premakumar & Rajagopalan, 1974; 1978), bismuth (Piotrowski & Szymanska, 1976; Szymanska, Mogilnicka & Kaszper, 1977), and zinc (Webb, 1972; Winge, Premakumar & Rajagopalan, 1974; Cherian, 1977). Various stressful conditions such as starvation (Bremner & Davies, 1975), cold exposure, strenuous exercise, CCl_4 intoxication (Oh, Deagan et al., 1978), and inflammation (Sobocinski, Canterbury et al., 1978) also were shown to induce the synthesis of zinc-thionein. These findings have suggested the involvement of MT in the homeostatic regulation of zinc metabolism. Other functions suggested for MT include heavy metal detoxification and poisoning-prophylaxis (Kimura, Otaki et al., 1974; Webb, 1975; Cherian & Goyer, 1978). Pretreatment of animals with low doses of cadmium prevented some of the toxic effects of a later higher dose (Nordberg, Piscator & Lind, 1971). Metal storage (Cherian, 1977), and transport (Weser, Rupp et al., 1973) also have been suggested as functions of MT.

The possibility of a gold-MT complex has been investigated only in the last 5 to 6 yrs. Gold chloride was shown to elevate the amount of a MT-like protein in rat kidney (Mogilnicka & Piotrowski, 1977; 1979), and rat liver cytosol (Winge, Premakumar & Rajagopalan, 1978). Gold(III) is considerably more toxic than gold(I) (Sabin & Warren, 1940; Libenson, 1945), thus, a non-specific stress-mechanism could be involved (Shaw, 1979; 1981) as has been reported for zinc-thionein (Bremner & Davies, 1975; Oh, Deagan et al., 1978; Sobocinski, Canterbury et al., 1978). However, studies with gold(I) drugs (ATM, ATG, triethylphosphine gold chloride), also observed a 10,000 molecular weight gold-binding protein in kidney (Thompson, Blaszak et al., 1978) and liver cytosol (Lawson, Danpure & Fyfe, 1977; Sharma & McQueen, 1980, Schmitz, Minkel et al., 1980). Although only a small amount of total gold (15-20%) was bound to this protein (Thompson, Blaszak et al., 1978) possibly indicating a low specificity for gold (Schmitz, Minkel et al., 1980), it was identified as MT because of its distinctive properties. These included: its exceptionally high cysteine content accounting for about 30% of the total amino acid residues, its ability to incorporate 7-8 mole of metal (gold, cadmium, zinc, copper) per mole of protein, its relative molecular weight (Kagi, Himmelhoch et al., 1974; Kagi, Kojima et al., 1980), and its heat stability (Webb, 1972; Shaikh & Smith, 1976; Cherian, 1977). The reaction of ATM with purified MT from horse kidney demonstrated unambiguously that gold was bound to this protein forming a gold-thionein complex (Schmitz, Minkel et al., 1980; Shaw, 1981). Gold(I)-MT was present in the kidney and liver cytosol within one-half hour of the administration of ATM (Sharma & McQueen,

1980) suggesting that gold is incorporated into formed MT. Turkall et al., (1977) also reported binding of gold to presynthesized MT, but prolonged treatment of mice with ATM alone did not induce synthesis of a MT-like protein. Thus it is unclear whether gold(I) is capable of stimulating MT synthesis. The observation of the binding of gold to MT, particularly in the kidney, suggests that MTs may have a role in the sequestration and localization of gold. It is possible that protection against gold toxicity might be conferred by pretreatment with low doses of gold and/or other MT-inducing agents such as zinc (Mason, Sharma & McQueen, 1981). This would account for the lower incidence of gold toxicity both in patients receiving low doses of gold, and in patients receiving oily suspensions of gold salts as opposed to more rapidly absorbed aqueous solutions (Lawrence, 1976). Another possibility that has been suggested is that gold acts by interfering with zinc-and-copper-metabolism (Schmitz, Minkel et al., 1980; Mason, Sharma & McQueen, 1981). Both copper and zinc have been implicated in the etiology of RA and may be involved in the mechanism of action of gold (Shaw, 1981).

b. LIVER

Within 24 hr. of the administration of ATM, gold was detectable in the liver. However, the amount was 14x less than in kidney tissue (Sharma & McQueen, 1980). The Kupffer cells preferentially accumulated gold (Yarom, Stein et al., 1975b; Vernon-Roberts, Dore et al., 1976) but the hepatocytes, because of their numbers contained most of the total gold (Penneys, McCreary & Gottlieb, 1976). Subcellular distribution studies in rat liver showed gold present in the cytosol

(Penneys, McCreary & Gottlieb, 1976; Lawson, Danpure & Fyfe, 1977; Mason, McQueen & Sharma, 1977; Turkall, Bianchine & Lerber, 1977; Schmitz, Minkel et al., 1980; Sharma & McQueen, 1980), lysosomes (Davies, Lloyd & Beck, 1971; Penneys, McCreary & Gottlieb, 1976; Lawson, Danpure & Fyfe, 1977), nuclei (Penneys, McCreary & Gottlieb, 1976; Mason, McQueen & Sharma, 1977), and mitochondria (Penneys, McCreary & Gottlieb, 1976; Abou-Khalil, Yunis & Abou-Khalil, 1981). Mason et al., (1977) and Lawson et al., (1977) noted a high concentration of gold in the cytosol at first; this rapidly declined over the next few days. The reduction in cytosolic gold was associated with an increase in gold in the lysosomal fraction. It has been suggested that this might account for the delayed therapeutic effect of the various gold compounds used in the treatment of RA (Penneys, McCreary & Gottlieb, 1976; Lawson, Danpure & Fyfe, 1977). The gold found in both the nuclear and mitochondrial fractions of liver, as in kidney, is believed to be in the form of aurosomes (Lawson, Danpure & Fyfe, 1979). Gel-permeation chromatography of the liver cytosol showed that gold was associated with 3 or 4 species similar to those found in kidney cytosol (Lawson, Danpure & Fyfe, 1977). It has been established that the l.m.w. gold-binding protein present in liver cytosol is MT (Sharma & McQueen, 1979; Schmitz, Minkel et al., 1980). Because gold has a strong preference for a linear 2-coordinate geometry, it has been postulated that the gold binds to a single sulphydryl group of MT with retention of the thiomalate carrier ligand, or, more likely, that it is chelated by two sulphydryl residues on MT with loss of the thiomalate moiety (Schmitz, Minkel et al., 1980).

c. SYNOVIAL TISSUE

After it was shown that gold salts were concentrated in inflamed areas (Bertrand, Waine & Tobias, 1948; Jeffrey, Freundlich & Bailey, 1958), investigators examined the joints and synovia of gold-treated animals (Tonna, Brecker et al., 1963; Norton & Ziff, 1966; Norton, Lewis & Ziff, 1968), and RA patients on chrysotherapy (Inoune, 1968; Gottlieb, Smith & Smith, 1972a; Grahame, Billings et al., 1974). After administration of ATM or other gold compounds, more gold was found in inflamed than unaffected joints (Lawrence, 1961; Kantor, Bishko et al., 1970; Sairanen & Vahatalo, 1973; Vernon-Roberts, Dore et al., 1976). This could result from the increased blood flow to the inflamed joints. Gold diffused rapidly into the synovial fluid and a steady state equilibrium was established within a few hours between gold in serum and synovial fluid (Freyberg, Block & Levey, 1941; Gerber, Paulus et al., 1972a; Grahame, Billings et al., 1974). In some reports, the gold concentrations were the same in synovial fluid and serum (Freyberg, Block & Levey, 1941) but in most, synovial concentrations were 50 to 60% of those in serum (Smith, Peak et al., 1958; Lawrence, 1961; Sliwinski, 1968; Kantor, Harley et al., 1971; Gerber, Paulus et al., 1972a). Synovial-fluid gold concentrations were lower during auranofin treatment than during parenteral chrysotherapy (Thomas & Ghadially, 1977). After conventional chrysotherapy, less gold was found in the joints than in the reticuloendothelial system, but the synovial fluid and membrane contained more gold than other articular tissue (Gottlieb, Smith & Smith, 1972a; Grahame, Billings et al., 1974). Gold deposits were identified in the lysosomes of type A synovial lining cells and the subsynovial macrophages by electron microscopy and electron probe x-ray analysis (Tonna, Brecker et al., 1963; Ghadially & Roy, 1967; Norton, Lewis & Ziff, 1968; Inoune, 1968; Ghadially, Oryschak & Mitchell, 1976; Oryschak & Ghadially, 1976a,b; Nakamura & Igarashi, 1977). The gold-containing lysosomes (aurosomes) had a characteristic appearance (Ghadially, Thomas & Lalonde, 1977) that was unaffected by the gold compound used (ATM, ATG, ATS) (Ghadially, Thomas & Lalonde, 1977), the route of administration (im, ia) (Oryschak & Ghadially, 1976a,b), the site of localization e.g. synovial membrane, kidney, liver, or skin (Yarom, Stein et al., 1975b; Lawson, Danpure & Fyfe, 1977; Ghadially, DeCoteau et al., 1978), and species (rat, rabbit, man) (Ghadially, 1979). Triethylphosphine gold chloride po induced aurosomes similar to those induced by other soluble gold compounds (Thomas & Ghadially, 1977). However, intra-articular injections of colloidal gold induced aurosomes of different morphology than those seen after soluble gold compounds (Yarom, Hall et al., 1973). Ghadially et al., (1976) suggested that gold entered pre-existing lysosomes. Besides gold, sulphur and phosphorus were detected in the lysosomes using energy dispersive x-ray analysis (Nakamura & Igarashi, 1977; Ghadially, Lalonde et al., 1978). Because the sulphur/gold ratio was higher than the ratio in ATM, it was suggested that binding of gold to MT occurred in the lysosomes (Nakamura & Igarashi, 1977). During chrysotherapy the gold lysosomal deposits became progressively denser with time. When chrysotherapy was stopped, the number of aurosomes in the synovium decreased (Nakamura & Igarashi, 1977) but they were still present in subsynovial macrophages (Vernon-Roberts, Dore et al., 1976) 2 yrs. after the last injection (Ghadially, Lalonde et al., 1978). These findings suggest that the beneficial therapeutic effects of gold salts may be mediated through an action on macrophages. There is evidence that gold salts not only suppress the activity of lysosomal enzymes of macrophages (Persellin & Ziff, 1966; Paltemaa, 1968), but also suppress their phagocytic activity (Vernon-Roberts, Jessop & Dore, 1973).

d. SKIN

Using NAA, trace amounts of gold were detected in skin, hair, and nails of normal adults not receiving gold compounds. During the first 3—12 weeks of chrysotherapy, gold concentration increased 2-5x (Gottlieb, 1977). Freyberg et al., (1942) reported that weekly doses of 10 to 100 mg of various gold compounds produced skin gold levels of 0—40 μg/g. Lawrence (1961) and Gottlieb, Smith and Smith (1972a) reported similar levels in patients receiving approximately the same total doses of gold. A correlation between skin gold concentration and total gold dose was observed despite variations among patients (Penneys, Kramer & Gottlieb, 1975). Patients on long-term chrysotherapy had high skin gold levels ranging from 20-80 μg/g after a cumulative total dose of 3-6 g of gold (Gottlieb, 1977). Patients with chrysiasis had even higher skin gold levels (50-140 μg/g) but they generally had received more gold (Jeffery, Biggs et al., 1975). Gold was found in the skin of patients with and without dermatitis (Lawrence, 1961). Biopsies of skin lesions and 'normal' skin from the same patient contained similar amounts of gold (Gottlieb, Smith et al., 1974b). In most studies no relationship was found between skin reactions and gold content, except for chrysiasis (Jeffery, Biggs et al., 1975). Studies of skin using a suction blister technique showed that gold was largely confined to the dermis (Penneys, Kramer & Gottlieb, 1975). Using a laser microprobe, electron-dense particles containing gold were detected in the lysosomes (i.e. aurosomes, Ghadially, DeCoteau et al., 1978) of dermal cells (Cox & Marich, 1973). The finding of low gold levels in the epidermis (Penneys, Kramer & Gottlieb, 1975) supports previous findings (Gottlieb, Smith et al., 1974b) that keratinous tissues have little affinity for gold. This is in contrast to heavy metals such as lead and arsenic which accumulate in keratinous tissues and reflect accurately the total body burden (Gottlieb, Smith et al., 1974b). Gold was detected in the skin of some patients as late as 20 yrs. after the discontinuation of chrysotherapy (Cox & Marich, 1973) indicating very slow mobilization of gold.

e. HAIR, NAILS AND OTHER TISSUES

In post-mortem specimens from a patient with RA who had received a total of 5 g of ATG, very low concentrations of gold were present in the hair (5 μg/g) and nails (2 μg/g) (Gottlieb, Smith & Smith, 1972a). In another study by Gottlieb (1974b) slight increases in both hair and nail gold concentrations were observed during chrysotherapy. These results however were inconsistent and it was concluded that they were of little value in assessing total body gold in individual patients. In a study involving chrysiasis patients (Jeffery, Biggs et al., 1975), considerably more gold was measured in nails (1-4 μg/g) than in hair (0.2-0.7 μg/g) contrary to the results of Gottlieb (1972a) but in agreement with the findings of Kamel et al., (1976a). Since most studies have involved only small groups, more patients need to be studied to confirm these results. It is generally accepted that hair and nails contain low levels of gold because of the low affinity of keratinous tissues for gold (Gottlieb, Smith et al., 1974b; Penneys, Kramer & Gottlieb, 1975). Small amounts of gold have also been found in the crystalline lens of the eye (Hashimoto, Maeda et al., 1972; Gottlieb & Major, 1978). This appears to be dose dependent, developing in approximately 40% of patients receiving more than 1500 mg of ATM. However, it does not cause visual disturbances and disappears when chrysotherapy is stopped (Hashimoto, Maeda et al., 1972; Gottlieb & Major, 1978).

4. METABOLISM

The first "metabolism" studies (Block, Buchanan & Freyberg, 1941; 1942; 1944; Freyberg, Block & Wells, 1942) were actually distribution studies because available methods were only capable of detecting gold and not the chemical compounds with which it might be combined. Only recently has the actual metabolism or biotransformation of gold compounds been investigated. Danpure and coworkers demonstrated that ATM bound to l.m.w. thiols (cysteine) and disulphides (cystine) in vitro (Danpure, 1976b; Danpure & Lawson, 1977) and suggested that similar, but probably more complex, reactions would occur in the body because of the numerous SH-containing proteins. Evidence supporting this suggestion was provided by Isab and Sadler (1976). Nmr studies showed the presence of mixed thiols in slow exchange reactions with cysteine, glutathione and other SH-containing compounds with the release of some of the bound thiomalate. Studies with ^{195}Au-^{14}C-thiomalate also demonstrated that gold and thiomalate separated rapidly in vivo. Twenty hours after the administration of double labelled ATM, 40% of the ^{14}C-thiomalate remained in the body, especially the kidney and liver, with the balance excreted in the urine (Jellum, Munthe et al., 1979; 1980). The gold moiety, which is widely distributed throughout the body, has only recently been isolated from urine as a protein-bound and a l.m.w. form (Schaeffer, Shaw et al., 1980). The protein-bound form has been identified as albumin but the l.m.w. complex has not yet been identified. It could be several substances including ATM or gold-sulphur complexes formed by reactions with endogenous thiols, e.g. aurocysteine or S-auroglutathione (Danpure, 1976b). Further work is in progress to identify this/these urinary gold metabolites. In other studies, a similar l.m.w. gold-complex was detected in serum (Ward, Danpure & Fyfe, 1977; Francois, Goldberg et al., 1978; Danpure, Fyfe & Gumpel, 1979), and in the cytoplasm of both kidney (Thompson, Blaszak et al., 1978) and liver cells (Lawson, Danpure & Fyfe, 1977). The serum-gold complex was at first believed to be "free" gold, or unchanged ATM, but it has since been suggested that gold is released back into the blood from tissues in the form of a l.m.w. thiol such as aurocysteine or gold-glutathione (Danpure, 1977) and that this/these metabolites may then be filtered by the kidney and excreted in the urine.

5. EXCRETION

Gold was detected in the urine and feces of both animals (Block, Buchanan & Freyberg, 1941; 1942; 1944) and patients on chrysotherapy (Hartung, Cotter & Gannon, 1941; Freyberg, Block et al., 1941; 1942) after the injection of various gold compounds. Considerable variation was observed, but usually more gold was excreted in urine than in feces (Gottlieb, 1977). Peak urinary excretion occurred within 24 hrs of the administration of gold and declined over the next week (Freyberg, Block et al., 1941; 1942; Bertrand, Waine & Tobias, 1948; Smith, Peak et al., 1958; Lawrence, 1961; Sliwinski, Zvaifler & Rubin, 1966; Mascarenhas, Granda & Freyberg, 1972; Harth, 1974; Leroy, Bureau et al., 1978). By contrast fecal gold excretion reached a maximum on the second or third day (Mascarenhas, Granda & Freyberg, 1972). Fecal gold content was variable and was affected by variations in intestinal transit time, bowel habits, frequency of elimination, and stool mass (Rencher & Beeler, 1969). During the first weeks of conventional chrysotherapy approximately 15% of the administered dose was excreted in the urine (Freyberg, Block & Levey, 1941; Smith, Peak et al., 1958; Lawrence, 1961). However, at steady state, 35-50% of the administered dose was excreted per week (Lorber, Atkins et al., 1969; 1973; Gottlieb, Smith & Smith, 1972b; Mascarenhas, Granda & Freyberg, 1972; Gerber, Paulus et al., 1972b), two-thirds of this being in the urine. Long term metabolic studies of a single dose revealed that 16% of the administered dose was excreted in the second week, 12% in the third week and 10% in the fourth week following injection (Gottlieb, Smith & Smith, 1972b). These results were confirmed by Mascarenhas et al., (1972) who reported that patients on maintenance therapy excreted an average of 77% of an administered dose per month. Because gold accumulates in the body on a weekly or even on a monthly dosage schedule, excretion of small amounts of gold in the urine and feces has been reported to continue for months after the last gold injection (Freyberg, Block & Levey, 1941). The retention of gold in, and its slow excretion from the body has been suggested as the reason for delayed toxicity in some patients.

The importance of fecal excretion of gold has been recognized only recently. Several investigators (Gottlieb, Smith & Smith, 1972b; Mascarenhas, Granda & Freyberg, 1972; Harth, 1974) have shown that fecal excretion accounts for 30% of total gold excreted, contrary to earlier reports suggesting that only small "insignificant" amounts of gold were excreted in feces (Freyberg, Block & Levey, 1941; Lawrence, 1961). It has been demonstrated that biliary secretion is involved in the elimination of heavy metals such as mercury (Norseth & Clarkson, 1971; Cherian & Vostal, 1973), lead (Klaassen & Shoeman, 1974), cadmium (Cikrt & Tichy, 1974), and platinum (Casper, Kelsen _et al._, 1979) in the feces. This may be the mechanism by which gold enters the gastrointestinal tract. However, one study involving a patient with biliary t-tube drainage indicated that less than 50% of the fecal gold came from bile (Kapelowitz, Nelp _et al._, 1964).

Following the administration of auranofin, 95% of the gold was excreted in the feces with only 5% eliminated in the urine (Gottlieb, 1979b). These findings are considerably different from those after parenteral chrysotherapy. Here, 70% of the injected dose is excreted in the urine and 30% in the feces (Gottlieb, Smith & Smith, 1972b; Mascarenhas, Granda & Freyberg, 1972). Incomplete absorption, absorption onto intestinal mucosal cells with subsequent shedding into the lumen, and secretion through the intestinal wall have been suggested as possible sources of the high fecal gold content after auranofin (Gottlieb, 1979b). Secretion via the biliary tract has also been suggested (Gottlieb, 1979b), but was not confirmed, nor was there any evidence of an enterohepatic circulation of gold (Weisman, Hardison _et al._, 1980).

Other routes of excretion of gold investigated include saliva

(Hartung, Cotter & Gannon, 1941), breast milk (Blau, 1973), and hair and nail (Gottlieb, Smith et al., 1974b). In an early study involving two patients who received approximately 500 mg of ATM, no gold was detected in the saliva (Hartung, Cotter & Gannon, 1941). Recent studies have not examined saliva or tears, so the concentration of gold in these is unknown. After a total dose of 135 mg of ATG administered post-partum, significant concentrations of gold were found in breast milk and trace amounts were detected in the nursing infant's blood (Blau, 1973), indicating that gold can pass from the maternal bloodstream into the milk and hence the child's serum. Only minute amounts of gold were excreted in the hair and nails (Gottlieb, Smith et al., 1974b) compared to urine and feces (Gottlieb, Smith & Smith, 1972b), demonstrating the insignificant excretory function of these tissues.

6. CORRELATION STUDIES

Many attempts have been made to correlate plasma and serum gold concentrations with therapeutic effect or toxicity. Some investigators have reported that plasma gold levels were higher in patients who responded to treatment or who experienced toxicity compared to non-responders or patients who encountered no side effects (Krusius, Markkanen & Peltola, 1970; Lorber, Atkins et al., 1973). However, most investigators found no relationship between gold concentration, therapeutic effect, and the development of toxicity (Silverberg, Kidd et al., 1970; Sharp, Lidsky et al., 1977; Van de Stadt & Abbo-Tilstra, 1980). Patients displaying a therapeutic response had similar serum gold levels to patients who did not respond. Also, no significant differences among serum gold levels were found in patients who experienced gold toxicity and those who did not (Mascarenhas, Granda & Freyberg, 1972; Gerber, Paulus _et al._, 1972b; Jessop & Johns, 1973; Rubinstein & Dietz, 1973; Gottlieb, Smith & Smith,1974a).

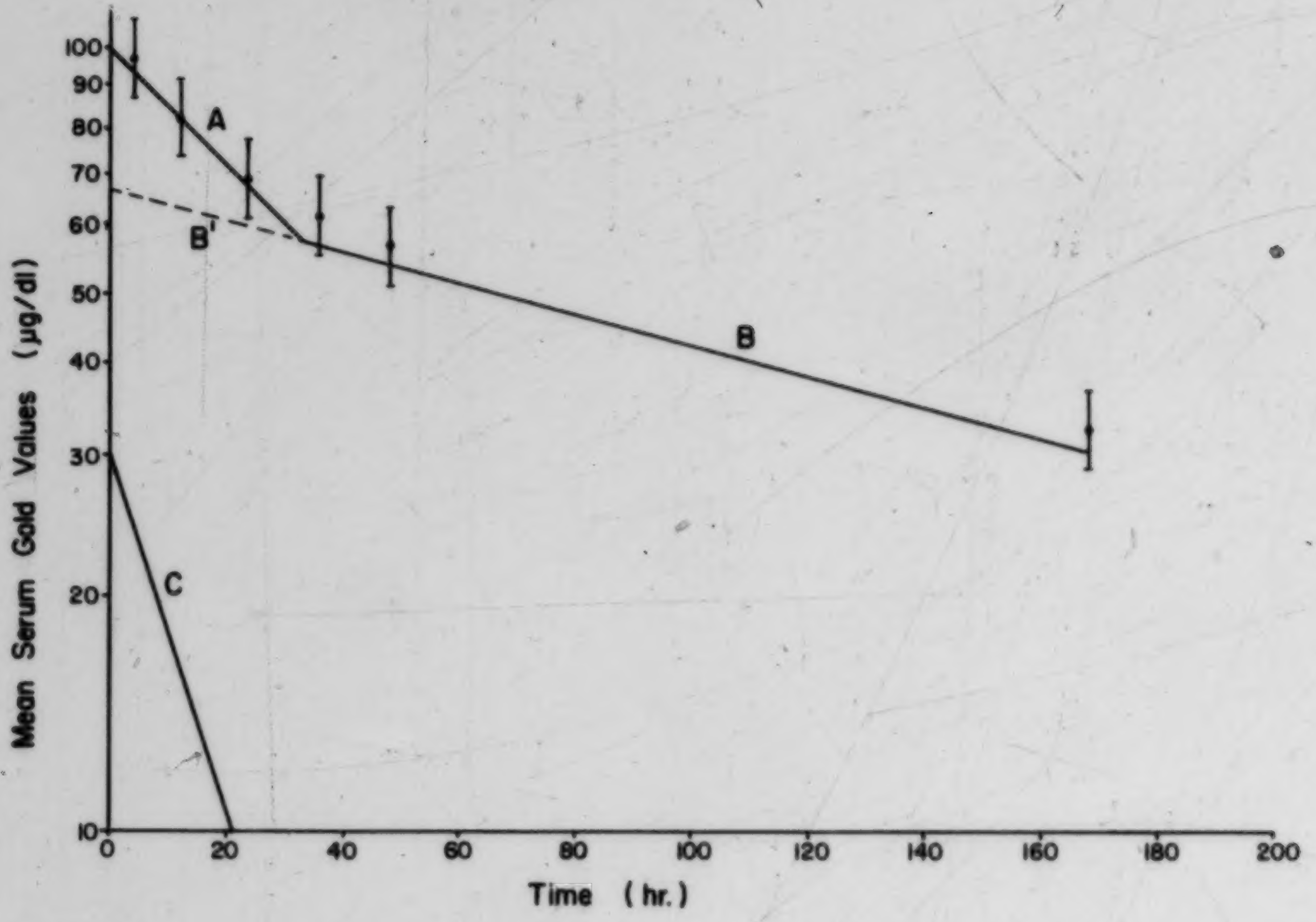
The relationships among gold excretion, therapeutic effect and toxicity have also been examined. Although some studies have indicated a correlation between urinary excretion rate (Smith, Peak _et al._, 1958; Krusius, Markkanen & Peltola, 1970; Leroy, Bureau _et al._, 1978), or fecal excretion rate (Gottlieb, Smith & Smith, 1972b) and therapeutic response, most studies have found no such relationship (Lawrence, 1961; Jones, Ahmed _et al._, 1971; Mascarenhas, Granda & Freyberg, 1972; Billings, Grahame _et al._, 1975; Sharp, Lidsky _et al._, 1977). Also, similar urinary, and total weekly excretion rates (Silverberg, Kidd _et al._, 1970; Gottlieb, Smith & Smith, 1972b) have been reported in patients experiencing toxicity and those who did not, contrary to the findings of Smith (1958) and Lawrence (1961).

7. KINETICS

Since the concentration of gold in body fluids did not correlate well with therapeutic response or toxicity, investigators began to study relationships among gold concentrations in different tissues. Various pharmacokinetic models have been used in an attempt to understand the distribution and excretion patterns of gold.

In most studies following administration of ATM, a biphasic decrease in serum gold concentration was observed (Figure 4; Harth, 1974). The initial decline in serum concentration comprises both the distribution of gold throughout body tissues and elimination. Later phases of the decline represent mostly elimination. A and B are

Figure 4. Semilogarithmic plot of serum gold disappearance curve after injection, showing a biphasic decline. (Harth, 1974)



regression lines drawn from observed values. Line C is obtained by the method of residuals (Gibaldi & Perrier, 1975). Using the above technique, serum half-lives ranged from 4.4 to 7.4 days (Gerber, Paulus et al., 1972a; Harth, 1974; Gottlieb, Smith & Smith, 1974a; Sharp, Lidsky et al., 1977). By contrast Walz et al. (1980), reported a half-life for blood and serum of 3 and 2.6 days respectively, after a single dose of ATM. Shorter half-lives (2.3 and 2.1 days, respectively) were observed following repeated administration (3 mg Au/kg/day,im for 4 days), indicating that the excretion rate can be altered during multiple dosing. In a 30-day study in two patients Waller et al. (1979) found the elimination half-life to be 9.7 and 10.4 days. Visual inspection of the terminal phase showed it became linear about 14 days after administration of ATM. Thus, 7 to 10-day studies may be too short to detect the true "terminal" excretion phase and may explain the differences among the elimination half-lives reported.

Waller et al. (1979) re-examined serum concentration—time curves from data gathered in single dose studies by Gottlieb et al. (1974a) and Palmer & Dunckley (1973) and found that elimination half-life increased with time. They concluded that studies must be conducted for an extended period of time in order to detect the terminal excretion phase. More recently a triphasic decline in serum gold concentration was observed after a subcutaneous injection of ^{195}Au-ATM in rats and terminal half-life calculated as 26.5 days (Mason et al., 1977c; 1979; 1981).

The pharmacokinetic behavior of auranofin, an orally active gold compound, was shown to be different from ATM (Walz, Griswold et al., 1980). After a single oral dose, blood and serum gold levels declined loglinearly. The half-life of gold was calculated as 1.8 days (blood) and 1.2 days (serum). The difference between blood and serum half-lives was attributed to extensive binding of auranofin to the formed elements of the blood. Multiple dosing did not alter the elimination half-lives significantly.

OBJECTIVES

From the literature review it will be apparent that gold is excreted mostly in the urine of animals and humans after administration of ATM. However, the importance of fecal gold excretion has recently been recognized. Biliary secretion is known to be involved in the fecal elimination of heavy metals such as cadmium, mercury, platinum and lead, and it may be the mechanism which results in significant amounts (30%) of gold being found in feces. Another possibility that has not been examined is the role of metallothioneins in the excretion of gold in feces. We decided to:

1. Examine the dose-dependency of the urinary and fecal excretion of gold given as sodium aurothiomalate (ATM).
2. Examine whether repeated administration of ATM influences the excretion of gold.
3. Determine the pharmacokinetic indices for the excretion of gold in urine and feces.
4. Examine the effect of the cholestatic agent alpha-naphthylisothiocyanate (ANIT) on the fecal elimination of gold, and
5. Examine the involvement of metallothioneins in the excretion of gold.

METHODS AND MATERIALS

A. IRRADIATION PROCEDURE

Radioactive ^{198}Au-labelled sodium aurothiomalate (ATM) was prepared by irradiation of ^{197}Au - ATM[a] according to reaction (1) (Chart of the Nuclides, 1977).

$^{197}\mathrm{Au} + n \rightarrow {}^{198}\mathrm{Au} + \gamma$ .... (1)

n = neutrons

γ = gamma rays of 412 KeV energy

$T_{1/2}$ = 64.7 hr for ^{198}Au

Samples were irradiated for 1 or 2 hr in a thermal neutron flux of $10^{12} n/cm^2 sec^{-1}$ in the University of Alberta SlowPoke nuclear reactor. Since the compound contains sodium, radioactive ^{24}Na was produced simultaneously according to reaction (2) (Chart of the Nuclides, 1977).

$^{23}\mathrm{Na} + n \rightarrow {}^{24}\mathrm{Na} + \gamma$ .... (2)

n = neutrons

γ = gamma rays of 1368 and 2754 KeV energies

$T_{1/2}$ = 15.0 hr for ^{24}Na

Following irradiation the samples were stored for a minimum of three days to allow most of the short-lived isotopes, especially ^{24}Na, to decay minimizing interference with the measurement of gold. Solutions of the drug were then prepared in 0.9% saline[b] for use in the animal experiments.

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[a] Lots M12777 and M15532, Poulenc Ltd., Montreal

[b] NaCl (Fisher Scientific Co., New Jersey) dissolved in demineralized, distilled water

B. ACUTE AND CHRONIC STUDIES

Groups of 4 male Sprague Dawley rats[c] weighing 250-550 g were used. A single dose of 0.5 mg or 1.0 mg Au/kg of ^{198}Au-ATM in a maximum volume of 0.2 ml of saline was injected[d] into the extensor quadriceps femoris muscles (Greene, 1955) of the hind leg of rats. For the chronic studies, "cold" ^{197}Au-ATM (1.0 mg Au/kg) was injected into alternate quadriceps muscles for 13 consecutive weeks with the last dose being ^{198}Au-ATM (1.0 mg Au/kg,im). After the administration of radioisotope, rats were placed in metabolism cages[e] and allowed food[f] and water[g] *ad libitum.*

Urine and fecal specimens were collected at 24 ± 4 hr intervals and analyzed for ^{198}Au activity. Samples collected 24 hr before injection, containing no gold, were the time zero controls. The ^{198}Au activity of all specimens was measured by counting the 412 KeV gamma emissions using a 3x3 inch NaI (Tl) well counter with an automatic sample changer[h]. The gamma spectrometer was set for an energy range of 390-440 KeV, well below the 511 KeV shoulder or peaks due to ^{24}Na. ^{24}Na interference and background radioactivity was negligible. The samples were counted with a statistical counting error of less than 5% (Early, Razzak & Sodee, 1975). To account for the decay of the radio-

[c] obtained from the University of Alberta Animal Services

[d] using a 26 G½ disposable needle (Becton-Dickinson & Co)

[e] Fisher Scientific Co.

[f] Lab-blox^{R} food pellets (Wayne)

[g] tap water

[h] Model 1185, Searle Automatic Gamma Counting System calibrated monthly so that 1KeV corresponded to 1 channel

isotope, corrections were made using equation (3) (Early, Razzak & Sodee, 1975).

$$A_t = A_o e^{-\lambda t} \quad \ldots\ldots (3)$$

where

A_t = the ^{198}Au activity (cpm) at time t (hr) obtained by counting the specimens

A_o = the ^{198}Au activity (cpm) injected into the rat

λ = the decay constant found by calculating $0.693/T\frac{1}{2}$

$T\frac{1}{2}$ = the half-life of the ^{198}Au isotope (64.7 hr)

t = the time (hr) elapsed since administration of ^{198}Au and the minus sign (-) indicates decreasing activity

For each rat the actual amount (μg) of gold excreted in the urine and feces during each 24 hr period was then calculated using equation (4).

$$\text{Amount } (\mu g) = \frac{D \times A_t}{A_o} \quad \ldots\ldots (4)$$

where

D = the dose administered (μg)

A_t = the ^{198}Au activity (cpm) in the urine or feces for each 24 hr period

A_o = the ^{198}Au activity (cpm) injected into the rat

To determine the kinetic parameters, urinary and fecal excretion rates were calculated for each 24 hr period and plotted on semilog paper (ordinate) vs the mid-time of each collection period (abscissa). Data were entered into the AUTOAN program (Sedman & Wagner, 1974) assuming first order elimination. Data were then entered into the NONLIN program (Metzler, 1968). A line was fitted through the last 3,4 and 5 data points. Using the correlation coefficients the best line was determined and the overall elimination rate constant (K) and $T\frac{1}{2}$ computed (Gibaldi & Perrier, 1975).

Student's t test and analysis of variance were used to determine statistical significance at the 95% level ($p < 0.05$). When appropriate, Duncan's multiple range test was performed (Snedecor & Cochran, 1967).

C. EXPERIMENTS WITH ANIT

The cholestatic agent alpha-naphthylisothiocyanate[i] (ANIT) was used to determine whether biliary secretion was involved in the fecal elimination of gold. Doses in excess of 150 mg/kg po were reported to cause complete biliary stasis within 18-24 hr (Griffiths, Rees & Sinha, 1961; Goldfarb, Singer & Popper, 1962), lasting from 3 to 5 days (Indacochea-Redmond & Plaa, 1971; Plaa & Priestly, 1976). Therefore, 24 hr before the administration of ^{198}Au-ATM (1.0 mg Au/kg,im) rats were gavaged[j] with ANIT in doses of 150 mg and 300 mg/kg. Because ANIT is insoluble in water (Merck Index, 1968), it was suspended in 1% carboxymethyl cellulose[k] (CMC) or dissolved in peanut oil[l]. Control animals were given CMC or peanut oil alone. Then the rats were placed in metabolism cages and urine and fecal samples collected at 24 ± 4 hr intervals and assayed for ^{198}Au activity as before.

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[i] Fisher Scientific Co.

[j] using a size 18 stainless steel animal feeding needle (Bio-Medical, Popper & Sons Inc., New Hyde Park, New York)

[k] Fisher Scientific Co.

[l] Planters

D. PRELIMINARY STUDIES OF METALLOTHIONEIN

1. ANIMAL EXPERIMENTS

A single injection or 14 consecutive weekly injections of ATM[a] (1.0 mg Au/kg) was/were administered intramuscularly into the hind leg(s) of rats (250-550 g) as before. 24 hr after the last injection, the rats were killed[m] and the kidneys and livers removed immediately. The renal cortex and any visible fatty tissue was cleared from the kidneys and livers which were then washed in chilled isotonic saline and weighed[n]. These tissues were either fractionated immediately using the method of Shaikh and Smith (1976) or were quick-frozen (-40°C) and analyzed later.

2. TISSUE PREPARATION

The procedure used to isolate MT was similar to the method of Shaikh and Smith (1976) (Figure 5). 5mM 2-mercaptoethanol[o] was added to the homogenates to maintain the reducing conditions normally found in the cellular environment and to prevent the oxidation of MT with redistribution of metals during isolation (Minkel, Poulsen et al., 1980). A 20% w/v homogenate of the kidney or liver tissue was prepared in 1mM Tris-HCl buffer[p], pH 8.6. Tissue was finely minced for 15 seconds in a Waring blender and then homogenized at

[m] rats had their necks broken after first being stunned by contact with a lead table.

[n] Mettler balance, Model H10 (Fisher Scientific)

[o] Eastman Organic Chemicals (Rochester, New York)

[p] gold label ultrapure grade (Aldrich Chemical Company, Inc., Milwaukee)

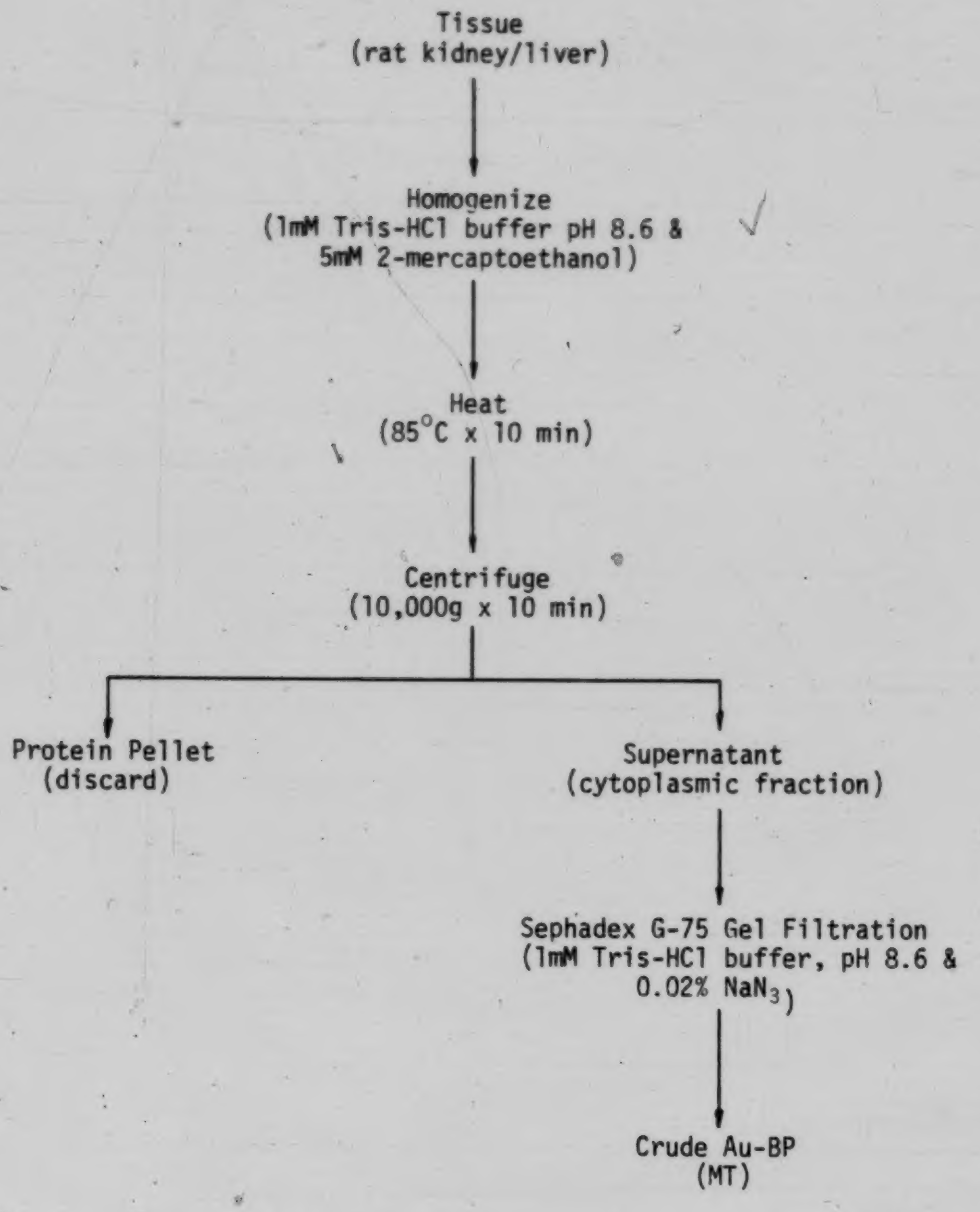

Tissue
(rat kidney/liver)
Homogenize
(1mM Tris-HCl buffer pH 8.6 &
5mM 2-mercaptoethanol)
Heat
(85°C x 10 min)
Centrifuge
(10,000g x 10 min)
Protein Pellet
(discard)
Supernatant
(cytoplasmic fraction)
Sephadex G-75 Gel Filtration
(1mM Tris-HCl buffer, pH 8.6 &
0.02% NaN_3)
Crude Au-BP
(MT)

Figure 5. Scheme for the isolation of Au-binding protein (Au-BP) believed to be MT (Shaikh & Smith, 1976).

$4^{\circ}C$ using a glass homogenizer[q] and teflon pestle. The homogenate was heated to $85^{\circ}C$ for 10 mins in a water bath[r] to coagulate h.m.w. proteins and to stop enzymatic reactions (Webb, 1972; Shaikh & Smith, 1976). Since MT is heat stable, it is not affected by this procedure. The homogenate was then cooled and centrifuged[s] at 10,000g for 10 mins at $4^{\circ}C$. The supernatant containing the MT fraction was collected and the protein pellet discarded. MT was then isolated from the supernatant using gel filtration chromatography.

3. COLUMN CHROMATOGRAPHY

10 ml of liver supernatant and 5 ml of kidney supernatant were applied respectively to 2.6 x 90 cm and 1.6 x 90 cm plastic columns packed with Sephadex G-75[t]. The samples were eluted from the columns with 1mM Tris-HCl buffer pH 8.6, containing 0.02% sodium azide[u] as a preservative. Using peristaltic pumps[v] the flow rates of the 2.6 x 90 cm and 1.6 x 90 cm columns were maintained at 28 ml/hr and 13 ml/hr ± 1 ml/hr respectively. 5 ml (liver) and 2.5 ml (kidney) fractions were collected[w] and their absorbance at 254 nm and 280 nm

[q] Tri-R-Stir-R, Model K43, 1/15 H.P., variable speed 50-1100 rpm

[r] Gallenkamp (England)

[s] Sorval Refrigerated Ultracentrifuge

[t] 40-120 micron particle size (Pharmacia Fine Chemicals)

[u] Fisher Scientific Co.

[v] LKB Model P3 and Watson-Marlow HR Flow Inducer

[w] LKB Ultrorac 7000 (Sweden) and Fractomette 200 Buchler Instruments (New Jersey)

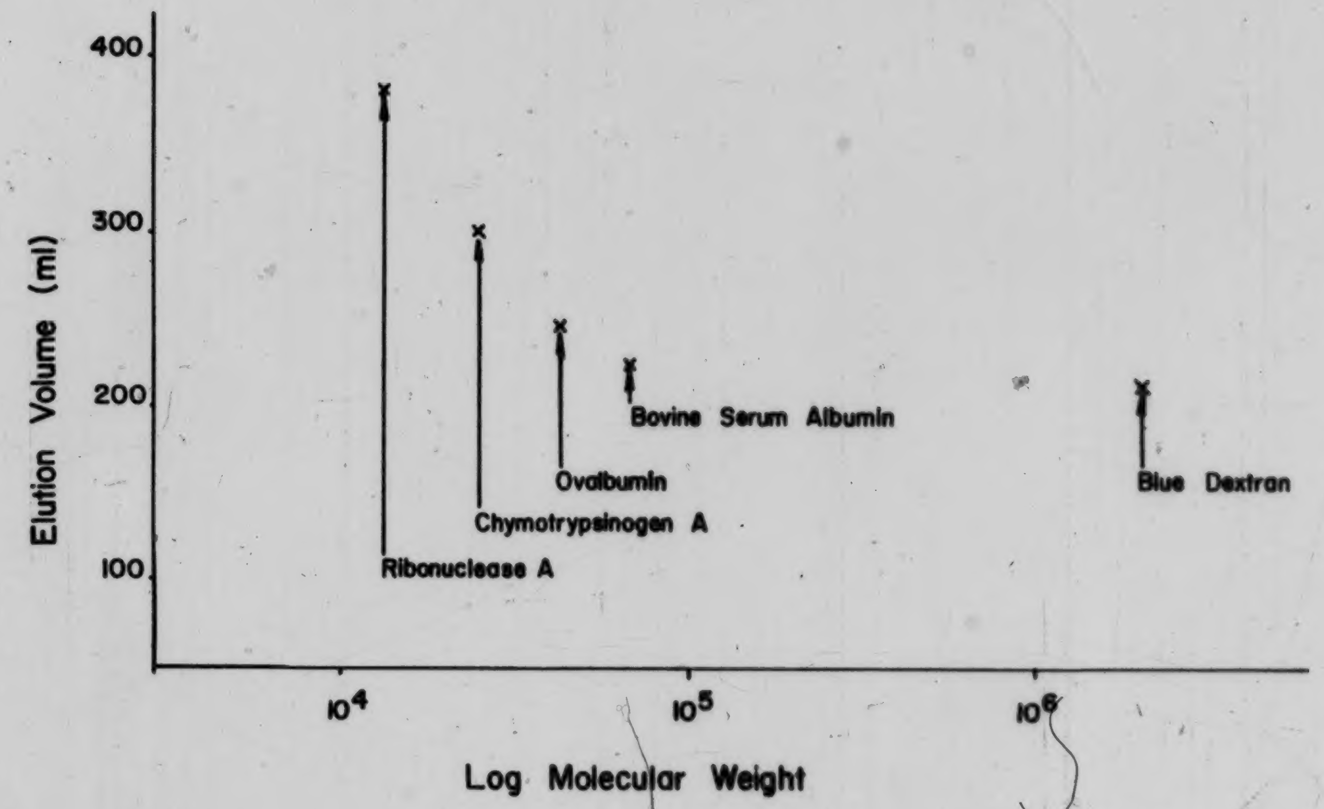
measured[x]. Protein concentration of each peak was measured by the colorimetric method[y] of Bradford (1976) and gold concentration was determined using NAA[z]. The columns were calibrated using blue dextran (MX 2 x 10^6), ovalbumin (43,000), chymotrypsinogen A (25,000) and ribonuclease A (13,700), and the MW of MT estimated using these markers (Figure 6) (Andrews 1964). Further fractionation of the crude MT or other tests to positively identify the l.m.w. gold-binding protein as MT were not performed.

[x] Gilford double beam spectrophotometer 250 or Unicam SP1800 UV Spectrophotometer

[y] The protein reagent containing Coomassie Brilliant Blue G250 (10 mg) (Serva, Heidelberg) was prepared as a solution containing 95% ethanol (5 ml), 85% w/v phosphoric acid (10 ml) (Fisher Scientific Co.), and demineralized distilled water (85 ml). One ml of protein reagent was added to test tubes containing 0.1 ml of protein solution (eluate) and the absorbance at 595 nm was measured. Using a calibration curve of absorbance values vs concentration of bovine serum albumin (2-20 μg/100 μl) (Sigma Chemical Company), the concentration of protein in each peak was determined.

[z] 5 μl samples of gold chloride standard (0.25-10 μg/ml concentration Harleco Gold Standard) in 0.1 mM Tris-HCl buffer, pH 8.6, and protein eluate samples were pipetted onto millipore filter discs (0.45 μM pore size, type HA, Millipore Corporation) and air dried. These discs were then packed into an irradiation capsule (standards interspersed among protein samples) and irradiated in the SlowPoke reactor for 1 hr in a thermal neutron flux of 10^{12} n/cm^2 sec^{-1}. After 3 days, the ^{198}Au activity was measured by counting the 412 KeV gamma emission using the Searle (Model 1185) Automatic Gamma Counting System. Based on the gold chloride calibration curve the concentrations of gold in the protein eluates was determined.

Figure 6 . Graph shows elution volume of five materials plotted against the logarithm of their MWs. Materials were separated on a Sephadex G-75 column (2.6 x 90 cm) using 1mM Tris-HCl buffer, pH 8.6.



RESULTS

A. ACUTE STUDIES

After the administration of single doses of ^{198}Au-ATM (0.5 mg and 1.0 mg Au/kg,im) the highest amounts of gold were found in urine during the first 24 hr. Amounts declined over the next 7 days. Fecal gold content reached a maximum by the second day and declined similarly over the next 7 days. Cumulative amount excreted/time plots are shown in Figures 7 and 8. In each 24-hr period, about twice as much gold was excreted in the urine and feces of rats given the 1.0 mg, as compared to 0.5 mg, Au/kg dose (Table IV). However, because of the small number of animals used and the large inter-animal variation, statistically significant differences were not observed for each time period. After 0.5 mg Au/kg, the total amount of gold excreted in the urine in 168 hr was 43.52 ± 7.21 μg (mean ± se, n = 4). This was significantly less than after the 1.0 mg Au/kg dose (92.78 ± 18.99 μg). The total amount of gold excreted in the feces after these two doses was not significantly different. About one third of the administered dose was recovered in the urine and feces over the 7-day period. Urinary excretion accounted for 71-74% of the gold excreted (Table V).

In rats given single injections of either 0.5 mg or 1.0 mg Au/kg a plot of rate of gold excreted in urine and feces vs time was curvilinear. Urinary and fecal excretion data vs time plots were obtained for each rat, and the apparent elimination rate constants calculated from the terminal portion of the graph and mean values obtained (Table VI). Plots of mean urinary and fecal data vs time are shown in Figures 9 and 10. Similarly K was calculated after the single dose of 1.0 mg Au/kg (Table VI).

Figure 7. The cumulative amount (mean ± se) of gold excreted in urine after a single injection of ^{198}Au-ATM,im (n=4). * $p < 0.05$

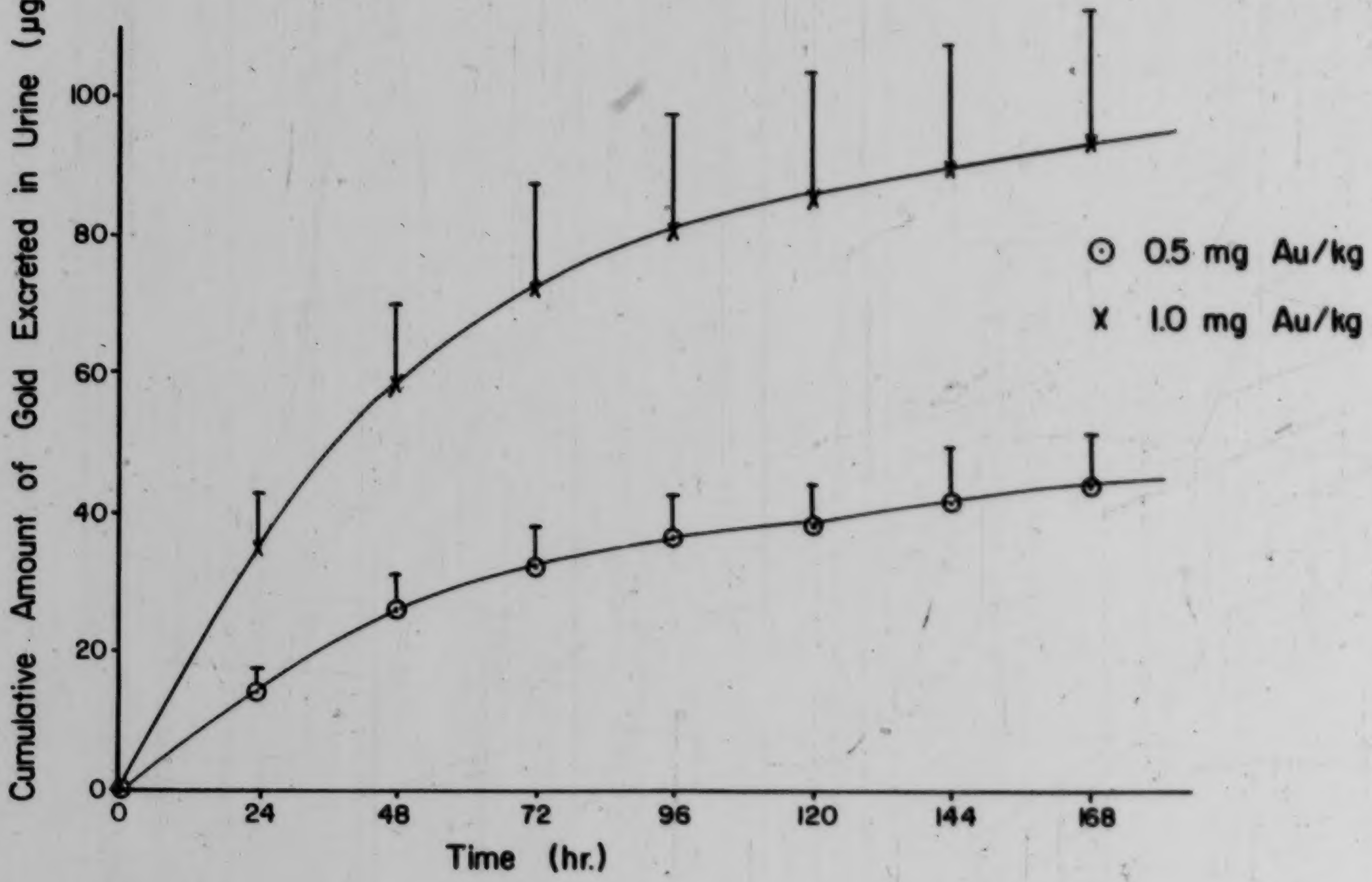


Figure 8. The cumulative amount (mean ± se) of gold excreted in feces after a single injection of ^{198}Au-ATM, im (n=4). * $p < 0.05$

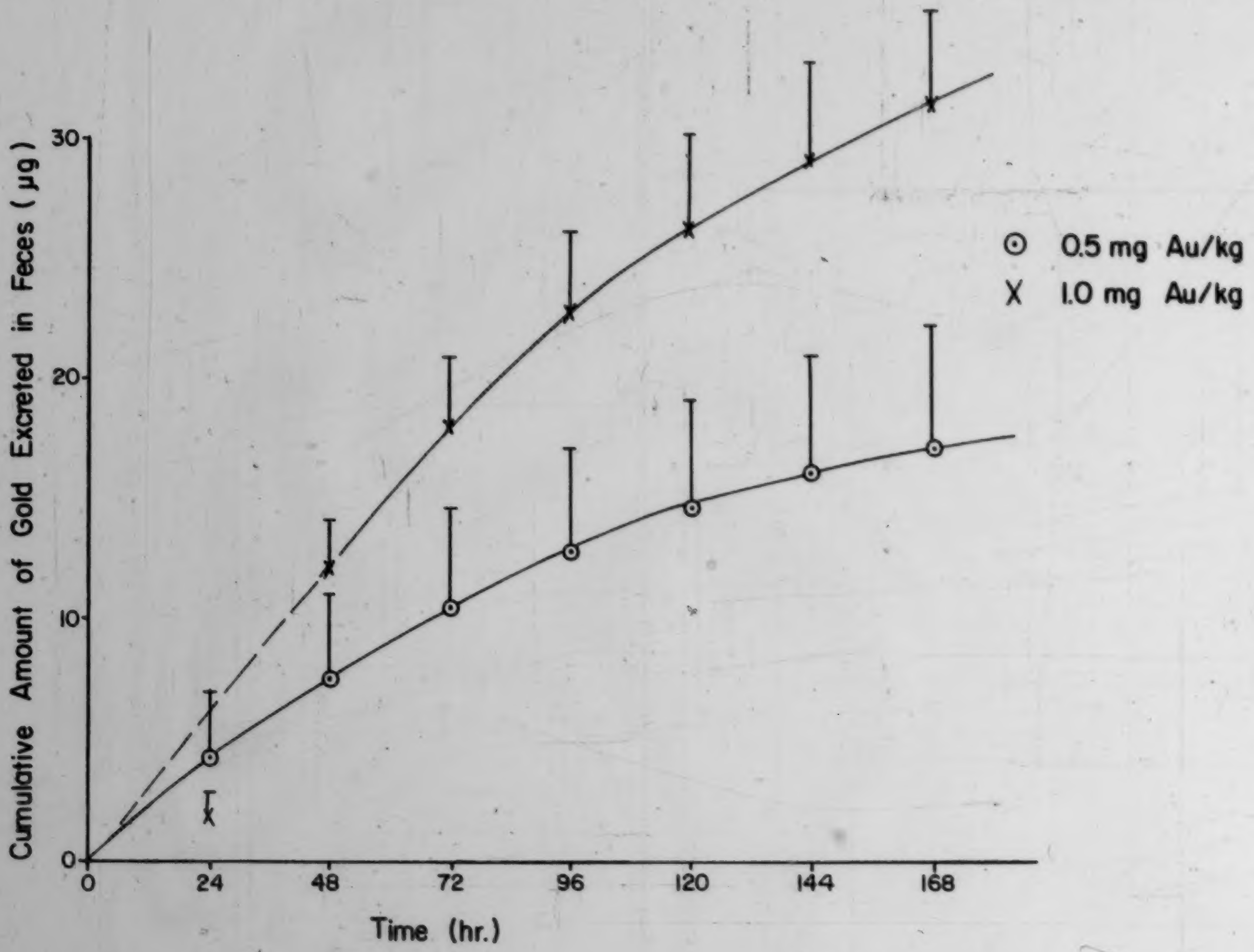


Table IV. The Amount (Mean ± SE) of Gold Excreted in Urine and Feces per 24 hr After a Single Injection of ^{198}Au-ATM, im (n=4). *p < 0.05

URINE

Dose		Time (hr)							Total
(mg Au/kg)		24	48	72	96	120	144	168	Excreted (µg)
0.5	Mean	13.82	12.24	5.98	4.46	2.71	2.28	2.05	43.52
	SE	3.02	2.32	0.83	0.63	0.46	0.29	0.13	7.21
1.0	Mean	35.11*	23.42	13.06	7.93	5.55	3.87	3.86*	92.78*
	SE	7.62	4.78	5.75	1.55	1.20	0.69	0.69	18.99

FECES

Dose		Time (hr)							Total
(mg Au/kg)		24	48	72	96	120	144	168	Excreted (µg)
0.5	Mean	4.22	3.22	3.11	3.28	1.83	1.33	1.12	17.12
	SE	2.77	0.98	0.44	0.36	0.24	0.21	0.13	4.88
1.0	Mean	1.67	10.58	5.85	4.61*	3.53	2.72*	2.42*	3.38
	SE	1.03	2.70	1.12	0.78	0.61	0.33	0.43	4.30

Table V. The Amount (Mean ± SE) of the Administered Dose of ^{198}Au-ATM Recovered in the Urine and Feces Expressed as a Percentage (n=4). * $p < 0.05$

Dose		Percentage of Dose Recovered		
(mg Au/kg)		Urine	Feces	Total
0.5	Mean	18.8	7.5	26.3
	SE	2.6	2.2	2.4
1.0	Mean	22.4	7.9	30.3
	SE	2.7	0.4	1.6
1.0 weekly	Mean	24.9	6.5	31.4
	SE	1.5	0.4	1.0

Table VI. Mean Apparent Elimination Rate Constants (K) of Gold (^{198}Au-ATM) in Rats Dosed Acutely and Chronically (n=4). * $p < 0.05$

		From Urinary Data	From Fecal Data
Dose (mg Au/kg)		K (hr^{-1})	K (hr^{-1})
0.5 acute	Mean	0.00942	0.01109
	SE	0.00063	0.00033
1.0 acute	Mean	0.00989	0.00899
	SE	0.00081	0.00068
1.0 chronic	Mean	0.01437	0.01212
	SE	0.00064	0.00085

Figure 9. Urinary excretion rate (mean ± se) of gold after a single injection of ^{198}Au-ATM, im (n=4).

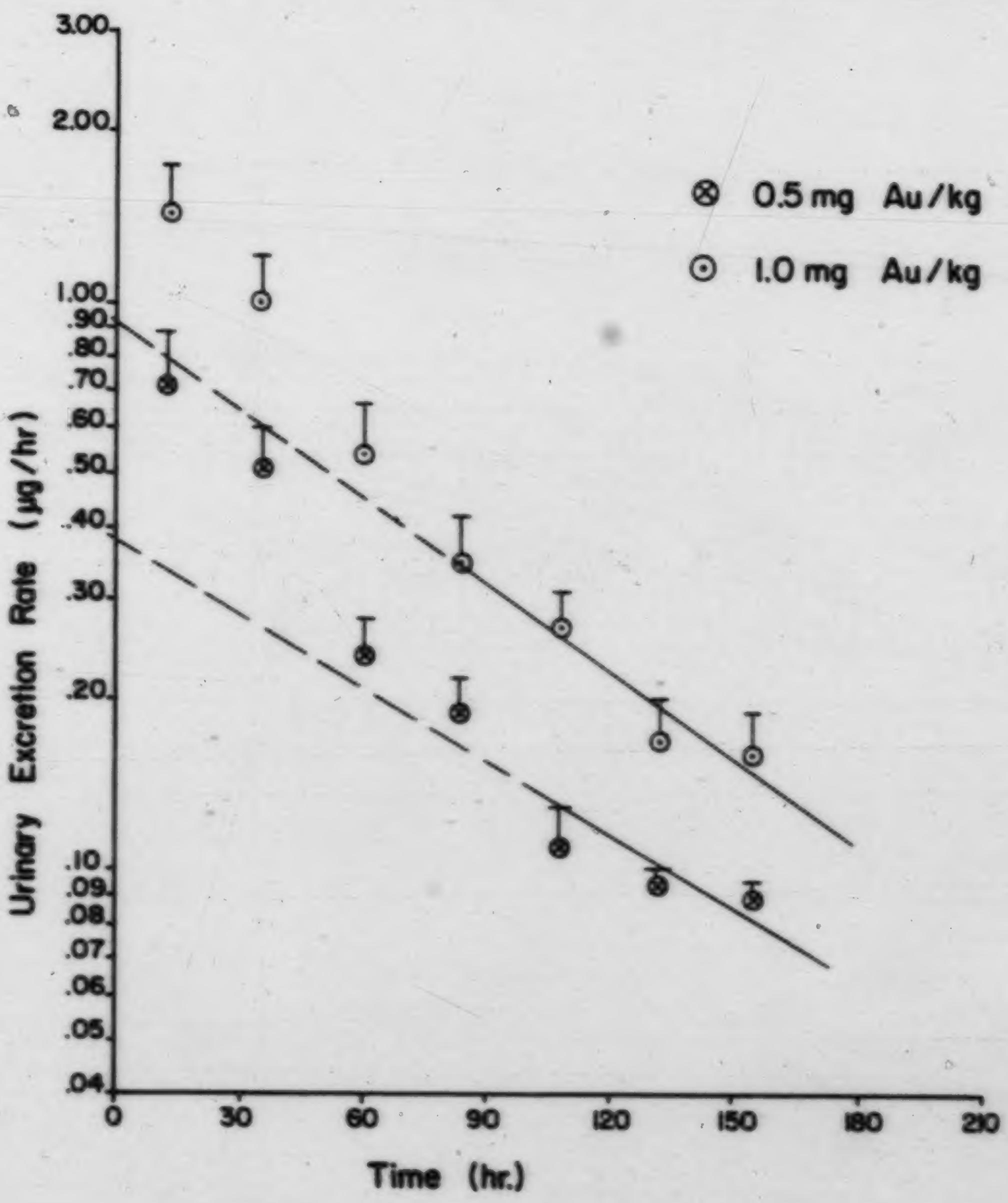
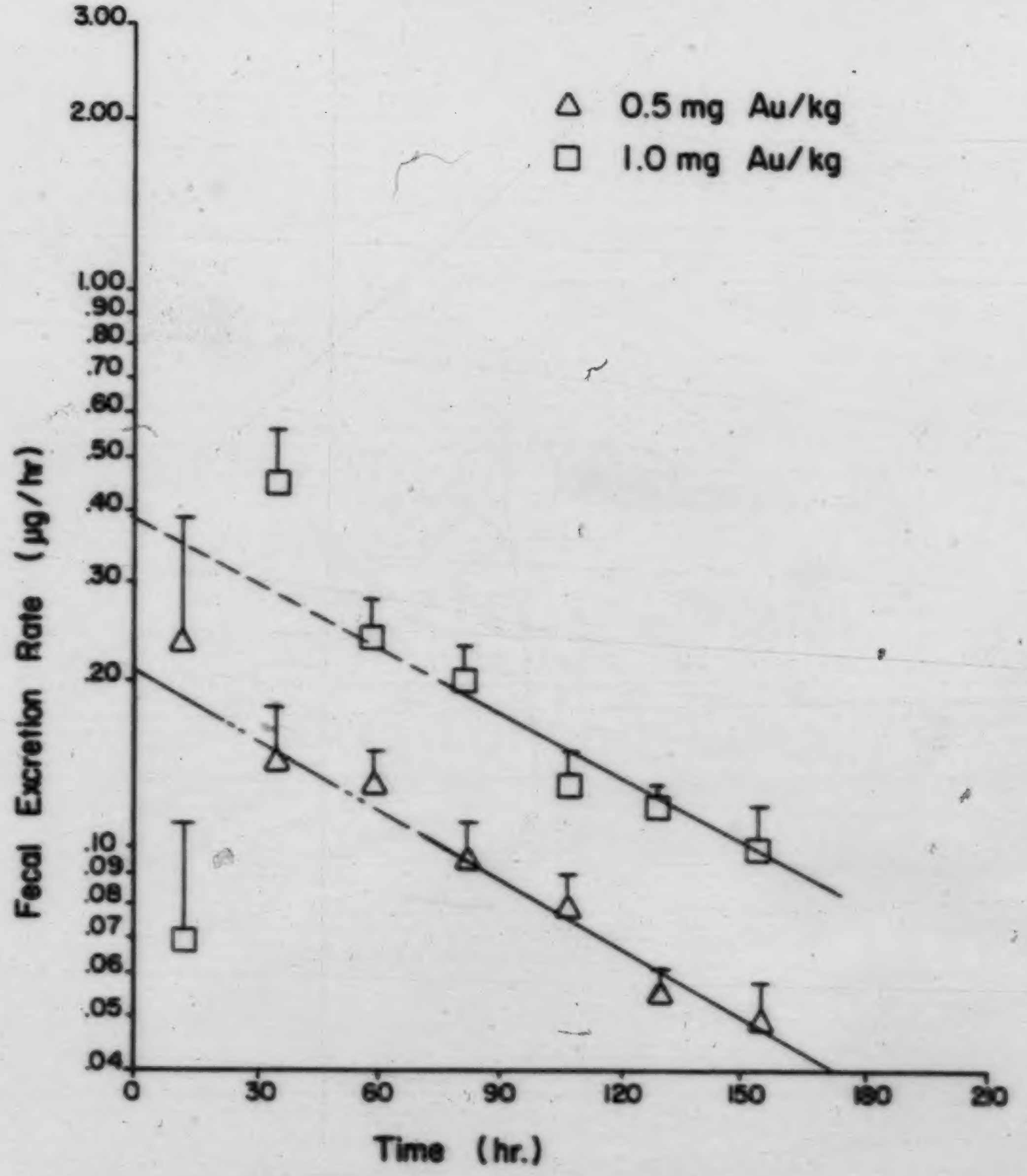


Figure 10. Fecal excretion rate (mean ± se) of gold after a single injection of ^{198}Au-ATM, im (n=4).



Data for individual rats are tabulated in Appendix A (Tables A1-A4).

B. CHRONIC STUDIES

After 13 weekly injections of ATM (1.0 mg Au/kg,im) and one injection of ^{198}Au-ATM (1.0 mg Au/kg,im), urinary and fecal excretion of ^{198}Au was similar to the acute studies. Urine specimens collected 24 hr after the injection of ^{198}Au-ATM contained the most gold and fecal gold content peaked on the second day. More gold was excreted in the urine and less in the feces for each 24 hr period but amounts were not significantly different from single dose studies (Table VII). The cumulative amounts of gold excreted per 7 day in the urine and feces were similar for both the acute and chronic studies (Figures 11 and 12; Table VII). As in the acute studies, one third of the administered dose was measured in the urine and feces after 7 days (Table V). Semilog urinary and fecal excretion-rate plots were curvilinear (Figures 13 and 14). Mean values of K for both urine and feces were significantly different from those from the 1 mg-acute studies (Table VI).

Data from individual rats are tabulated in Appendix A (Table A1-A4).

C. EXPERIMENTS WITH ANIT

Administration of ANIT in CMC and peanut oil 24 hr before ATM injection appeared to cause jaundice as judged by the yellowing of the sclera and feces, and the rats' lethargic behavior. Patterns of the excretion of urine were similar in test and control animals, but

Table VII. The Amount (Mean ± SE) of Gold Excreted in Urine and Feces per 24 hr After a Single 1.0 mg Injection and the 14th weekly 1.0 mg Injection of ^{198}Au-ATM, im (n=4). *$p \leq 0.05$

URINE

Dose (mg Au/kg)		Time (hr) 24	48	72	96	120	144	168	Total Excreted (μg)
1.0 single	Mean	35.11	23.42	13.06	7.93	5.55	3.87	3.86	92.78
	SE	7.62	4.78	5.75	1.55	1.20	0.69	0.69	18.99
1.0 weekly	Mean	42.17	30.46	13.35	9.61	5.91	4.61	3.01	109.11
	SE	2.98	2.13	0.79	0.55	0.24	0.16	0.12	0.70

FECES

Dose (mg Au/kg)		Time (hr) 24	48	72	96	120	144	168	Total Excreted (μg)
1.0 single	Mean	1.67	10.58	5.85	4.61	3.53	2.72	2.42	31.38
	SE	1.03	2.70	1.12	0.78	0.61	0.33	0.43	4.30
1.0 weekly	Mean	3.35	8.30	5.38	4.69	2.85	2.50	1.71	28.76
	SE	0.67	0.76	0.39	0.39	0.09	0.23	0.07	1.38

Figure 11. The cumulative amount (mean ± se) of gold excreted in urine after a single 1.0 mg injection (·) and the 14th weekly 1.0 mg injection (x) of ^{198}Au-ATM, im (n=4).

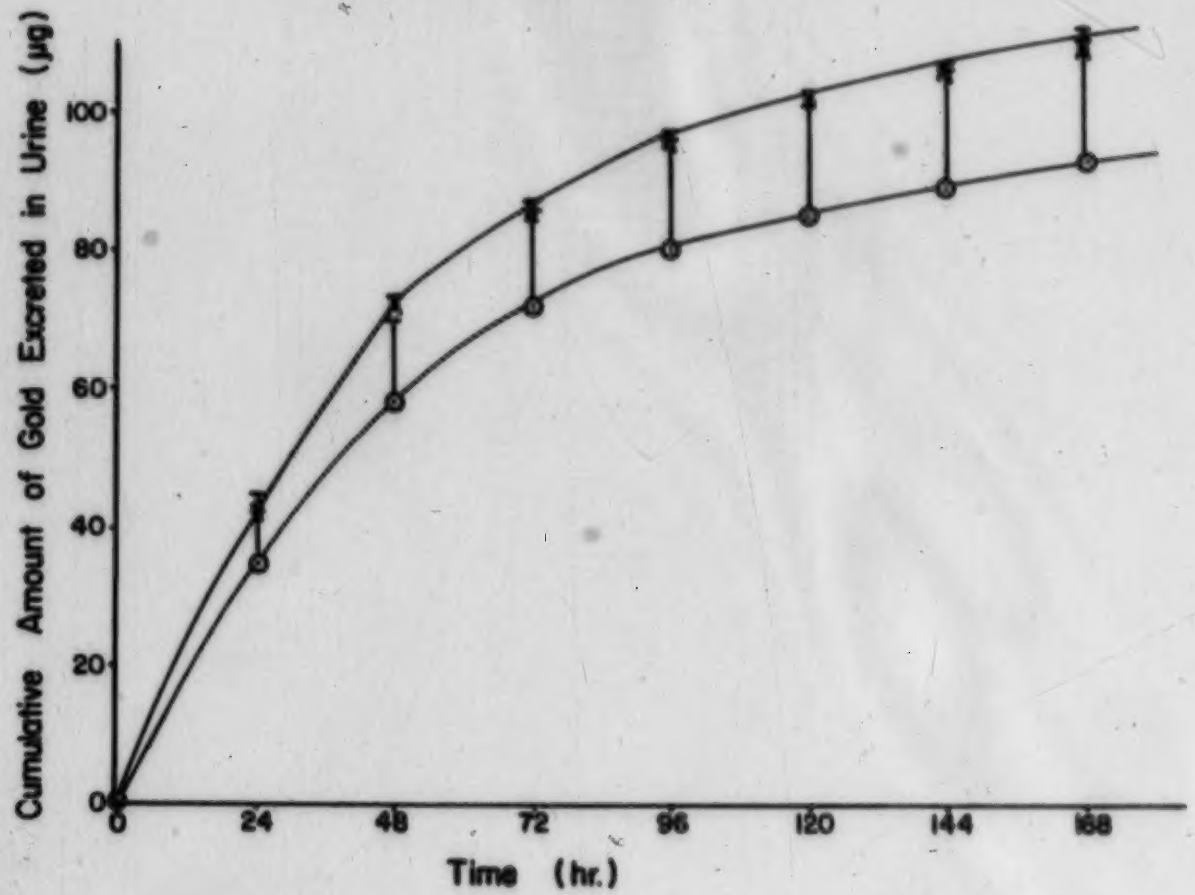


Figure 12. The cumulative amount (mean ± se) of gold excreted in feces after a single 1.0 mg injection (·) and the 14th weekly 1.0 mg injection (x) of ^{198}Au-ATM, im (n=4).

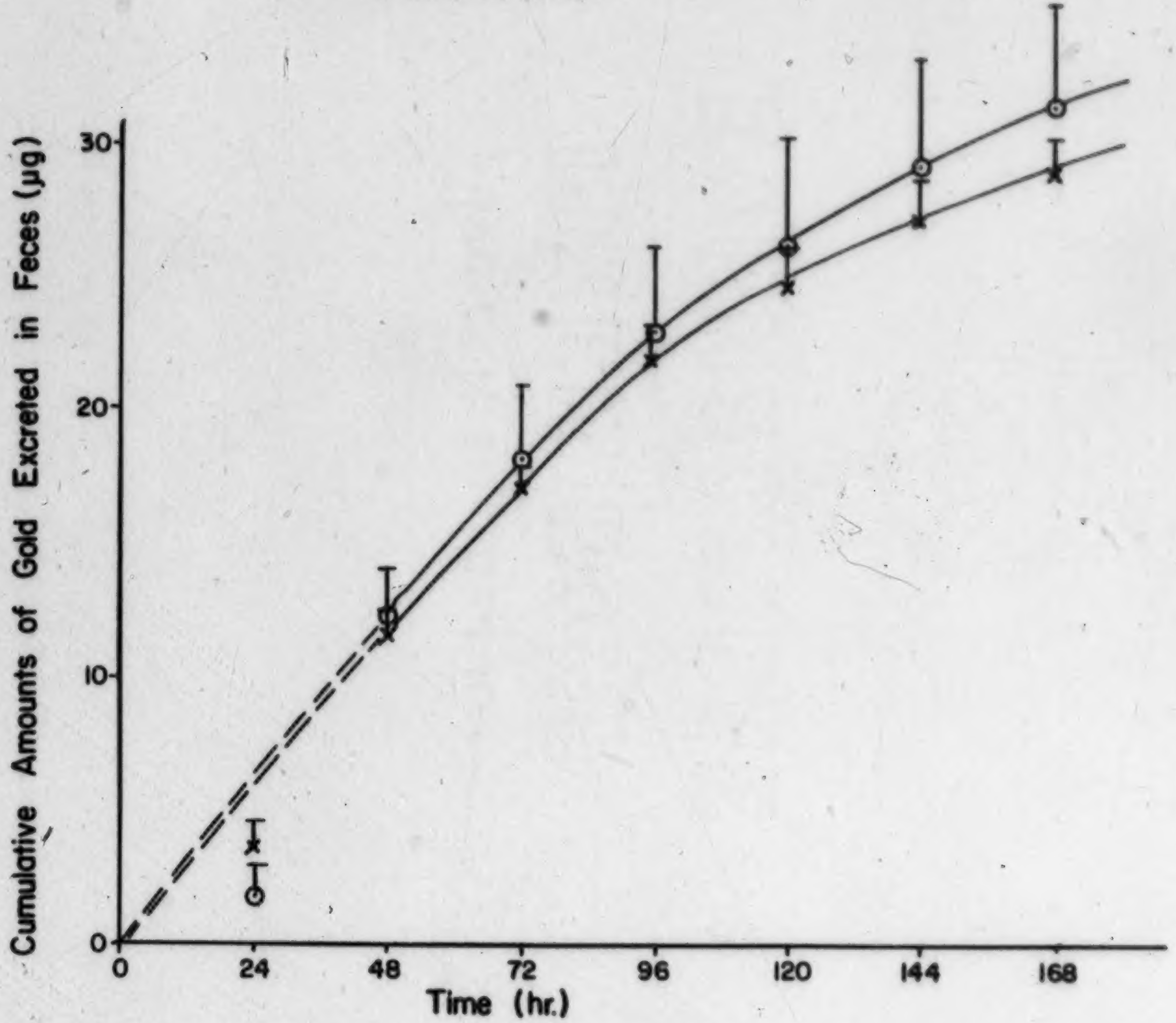


Figure 13. Urinary excretion rate (mean ± se) of gold after a single 1.0 mg injection (·) and the 14th. weekly 1.0 mg injection (x) of ^{198}Au-ATM, im (n=4).

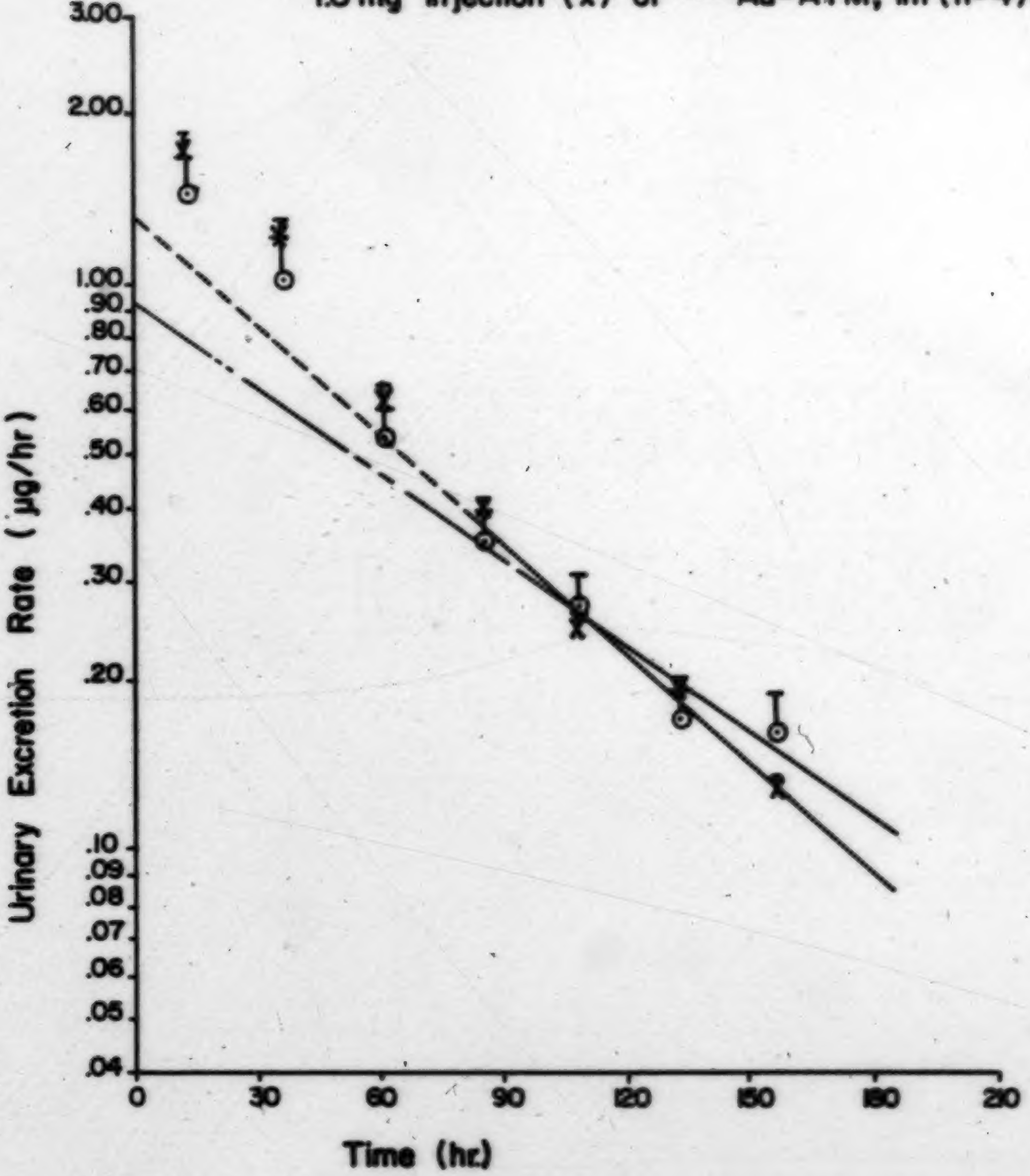
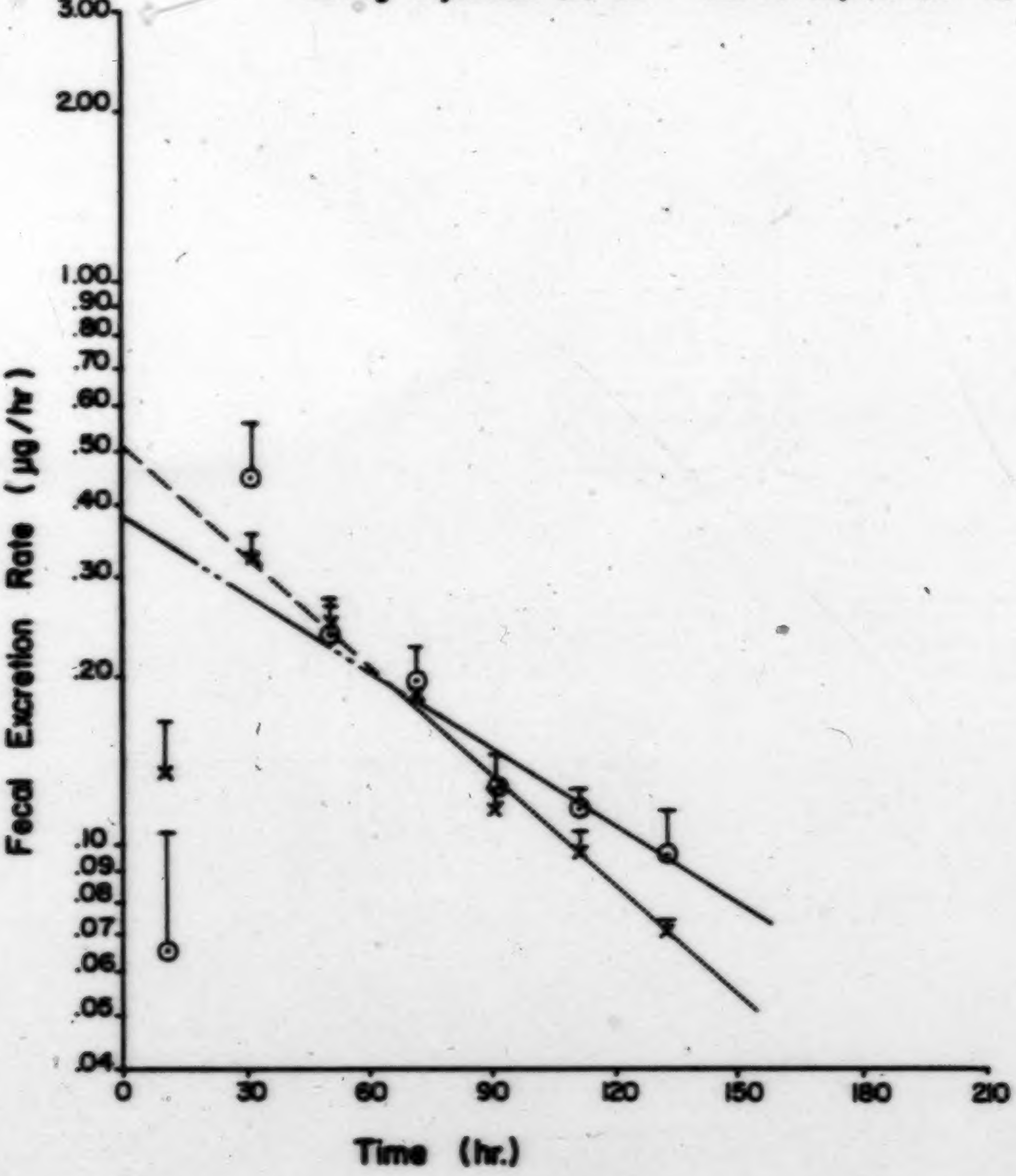


Figure 14. Fecal excretion rate (mean ± se) of gold after a single 1.0 mg injection (·) and the 14th. weekly 1.0 mg injection (x) of ^{198}Au-ATM, im (n=4).



the excretion of feces fell significantly in the first 72 hr after administration of ANIT. Urine contained the most gold 24 hr after the administration of ATM and declined over the 7 day study period. Although fecal excretion was erratic, fecal gold content generally reached a maximum by the second day. Cumulative amount excreted/time plots after ANIT in CMC and peanut oil are shown in Figures 15-18. ANIT pretreatment did however alter the total amount of gold excreted in the first 24-48 hr after ATM administration. During this period, 8.4% more gold was excreted in the urine and 11.5% less (both significant, $p < 0.05$) in the feces of ANIT-treated rats than controls that received only CMC or oil (Tables VIII and IX). After 72 - 96 hr, ANIT-treated and control rats excreted similar amounts of gold in the urine and feces. The total amount of gold excreted after 168 hr was also similar for ANIT-treated and control animals (Tables VIII and IX). As in the acute studies, only 27-32% of the administered dose of gold was recovered after one week — 73-76% in the urine and the remainder in the feces (Table X). Rats given ANIT (300 mg/kg,po) before injection of ATM excreted slightly more of the dose of gold in the urine (79%) but significantly less in the feces (21%). Figures 19 and 20 show that these rats defecated significantly less excreta in the first 72 hr than did controls. During this same period, rats given ANIT (150 mg/kg po) defecated more than those who had received the 300 mg/kg dose but again this was significantly less than controls (Table XI). Differences were observed between the vehicles CMC and oil but these were not statistically significant.

As in acute studies, semilog plots of urinary excretion rates (Figures 21 and 22) and fecal excretion rates (Figures 23 and 24)

Figure 15. The cumulative amount (mean ± se) of gold excreted in urine after administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in rats given ANIT in carboxymethylcellulose 24 hr beforehand (n=4). * $p < 0.05$

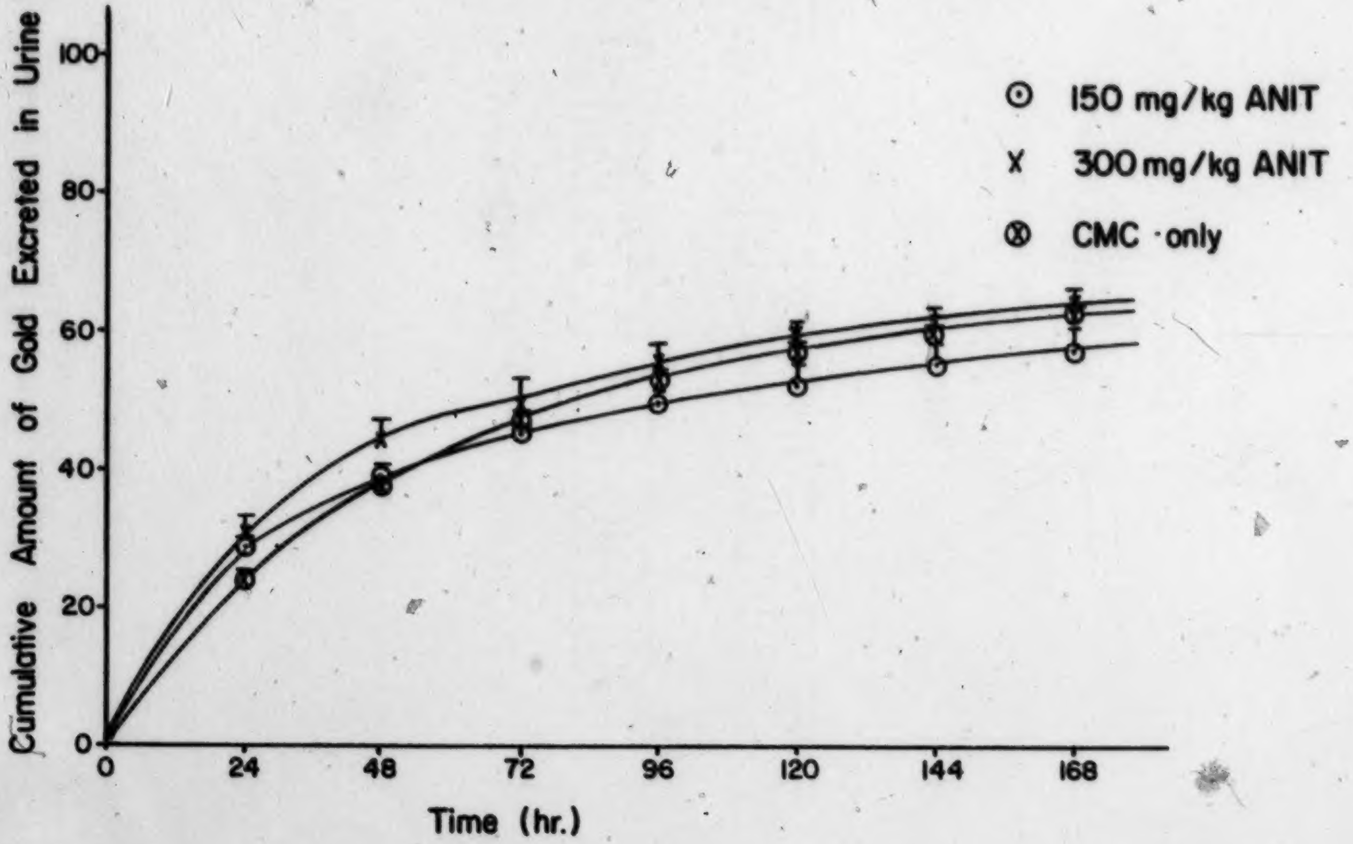


Figure 16. The cumulative amount (mean ± se) of gold excreted in urine after administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in rats given ANIT in peanut oil 24 hr beforehand (n=4). * $p < 0.05$

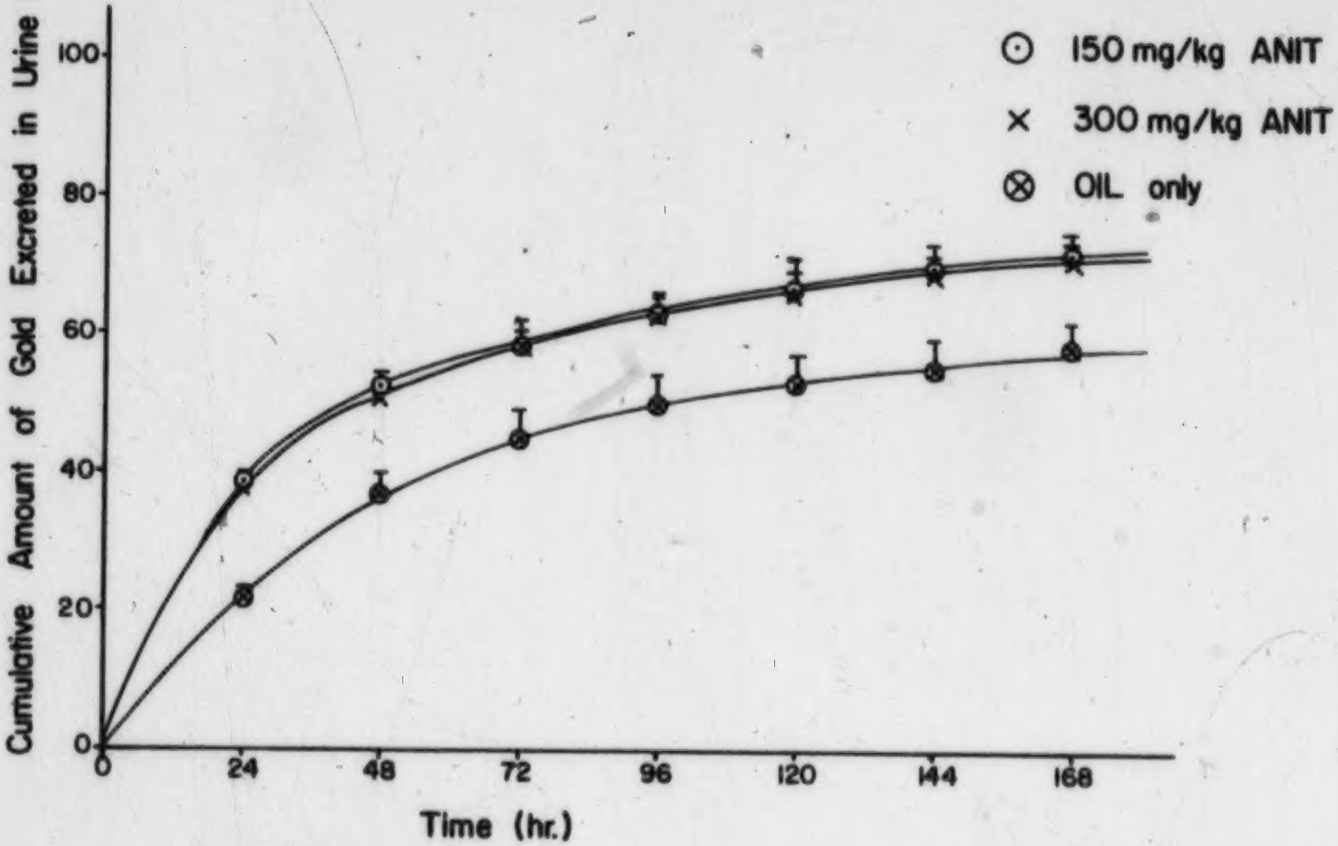


Figure 17. The cumulative amount (mean ± se) of gold excreted in feces after administration of ^{198}Au-ATM (1.0 mg Au/kg, im) in rats given ANIT in carboxymethylcellulose 24 hr beforehand (n=4). * $p < 0.05$

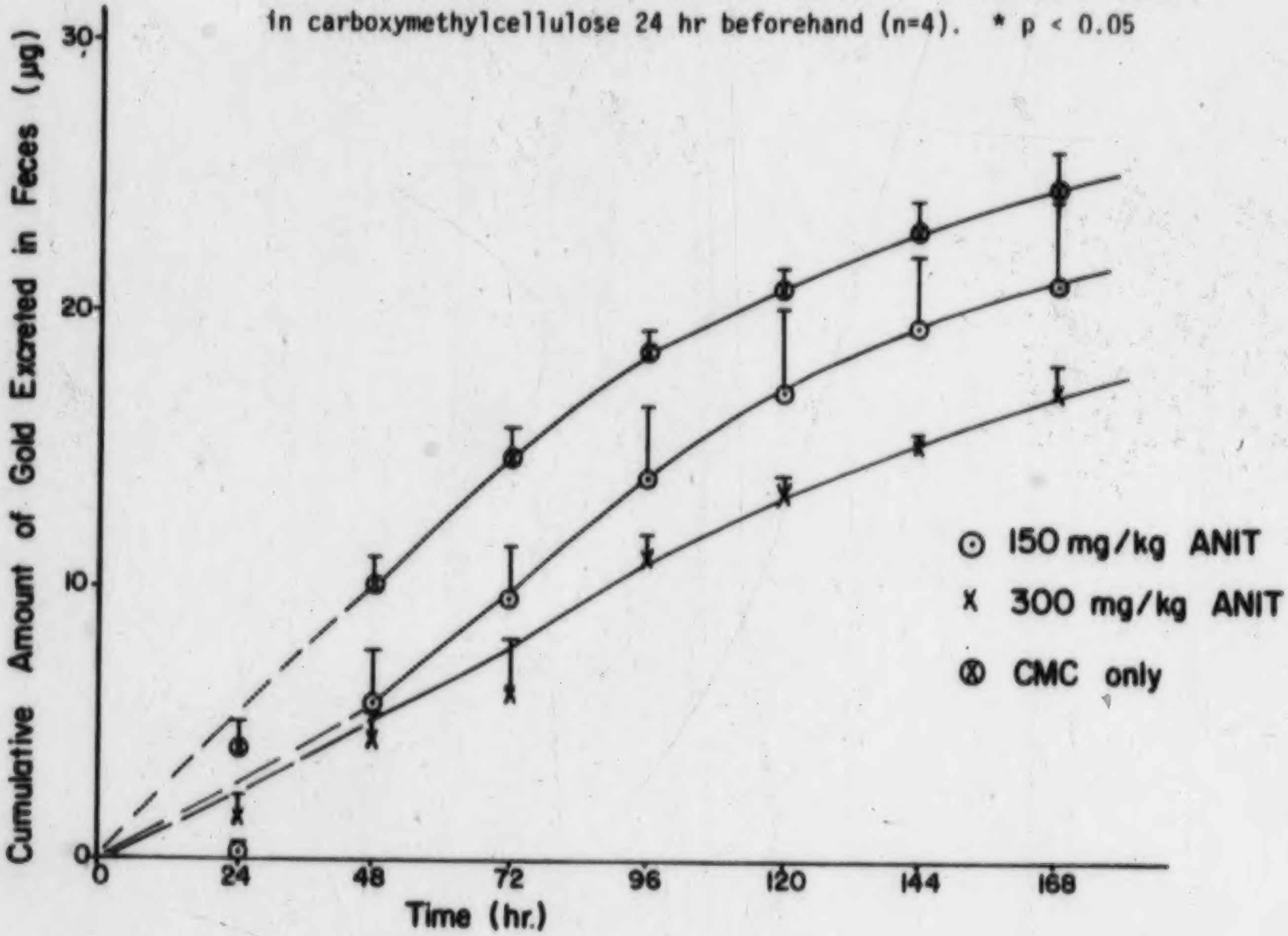


Figure 18. The cumulative amount (mean ± se) of gold excreted in feces after administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in rats given ANIT in peanut oil 24 hr beforehand (n=4). * $p < 0.05$

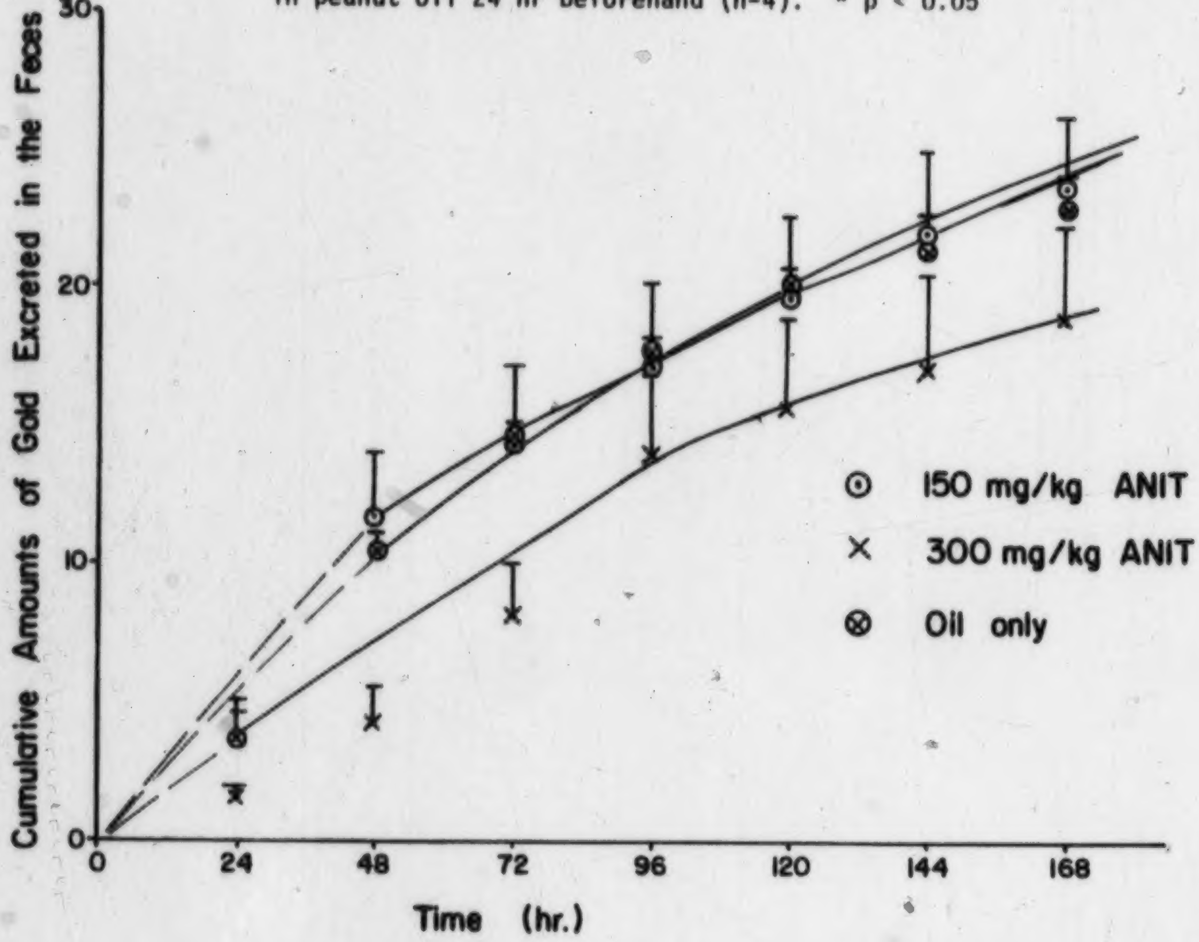


Table VIII. The Amount (Mean ± SE) of Gold Excreted in Urine and Feces After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im), in Rats Given ANIT in Carboxymethyl Cellulose 24 hr Beforehand (n=4). *$p < 0.05$

URINE

Dose (mg/kg ANIT)		Time (hr) 24	48	72	96	120	144	168	Total Excreted (μg)
150	Mean	28.85	10.56	5.52*	4.00	3.42	2.56	2.34	57.24
	SE	1.21	0.65	0.64	0.78	0.45	0.52	0.38	3.36
300	Mean	31.17	13.26	5.76*	5.32	3.28	2.18	2.86	63.83
	SE	3.62	1.51	0.82	0.90	0.18	0.39	0.36	2.55
CMC	Mean	24.06*	14.06	8.88	5.90	3.77	2.99	2.67	62.33
	SE	1.54	1.98	0.59	0.54	0.54	0.33	0.44	3.21

FECES

Dose (mg/kg ANIT)		Time (hr) 24	48	72	96	120	144	168	Total Excreted (μg)
150	Mean	0.37*	5.26	3.90	4.44	3.05	2.29	1.67	20.97
	SE	0.19	1.93	1.26	0.67	0.41	0.22	0.15	3.20
300	Mean	1.55*	2.78*	1.78	4.91	2.38	1.56	2.06	17.01
	SE	0.73	1.02	0.66	2.52	0.22	0.28	0.49	0.96
CMC	Mean	4.06	5.69	4.88	3.80	2.37	2.13	1.65	24.57
	SE	0.82	0.36	0.56	0.30	0.28	0.29	0.27	1.23

Table IX. The Amount (Mean ± SE) of Gold Excreted in Urine and Feces After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im), in Rats Given ANIT in Peanut Oil 24 hr Beforehand (n=4). *$p < 0.05$

URINE

Dose		Time (hr)							Total
(mg/kg ANIT)		24	48	72	96	120	144	168	Excreted (µg)
150	Mean	38.46	13.88	6.02*	4.60	3.45	2.44	2.62	71.47
	SE	0.93	1.21	0.36	0.15	0.21	0.24	0.06	2.58
300	Mean	38.67	12.33	7.61	4.03	2.62	2.62	2.26	70.13
	SE	1.58	0.95	1.55	0.63	0.35	0.32	0.29	5.28
Oil	Mean	22.25*	14.88	7.72	5.02	3.18	2.57	2.21	57.81
	SE	1.84	1.44	0.61	0.61	0.22	0.13	0.28	4.45

FECES

Dose		Time (hr)							Total
(mg/kg ANIT)		24	48	72	96	120	144	168	Excreted (µg)
150	Mean	3.47	8.18	2.70	2.73	2.52	2.15	1.70	23.43
	SE	1.77	1.28	0.52	0.29	0.25	0.11	0.12	3.09
300	Mean	1.48*	2.76*	3.87	4.30	1.61	1.58	1.38	18.46
	SE	0.47	1.28	1.30	2.50	0.43	0.15	0.20	3.48
Oil	Mean	3.67	6.74	3.98	3.19	2.03	1.74	1.38	22.73
	SE	1.05	0.78	0.40	0.37	0.07	0.26	0.04	1.25

Table X. The Amount (Mean ± SE) of the Administered Dose of ^{198}Au-ATM (1.0 mg Au/kg) Recovered in Urine and Feces (Expressed as a Percentage) in Rats Given ANIT in Peanut Oil and Carboxymethyl Cellulose (n=4). * $p < 0.05$

ANIT IN OIL

Dose		Percentage of Dose Recovered		
(mg/kg ANIT)		Urine	Feces	Total
150	Mean	24.1	7.8	31.9
	SE	1.1	0.9	1.0
300	Mean	23.1	6.0*	29.1
	SE	1.3	1.0	1.2
Oil	Mean	20.0	7.9	27.9
	SE	1.2	0.3	0.8

ANIT IN CMC

Dose		Percentage of Dose Recovered		
(mg/kg ANIT)		Urine	Feces	Total
150	Mean	19.9	7.3	27.2
	SE	0.5	1.1	0.8
300	Mean	21.8	5.7*	27.5
	SE	2.1	0.3	1.2
CMC	Mean	19.8	7.8	27.6
	SE	1.4	0.4	0.9

Figure 19. Weight (mean ± se) of feces excreted by rats given ANIT in carboxymethylcellulose (n=4). * $p < 0.05$

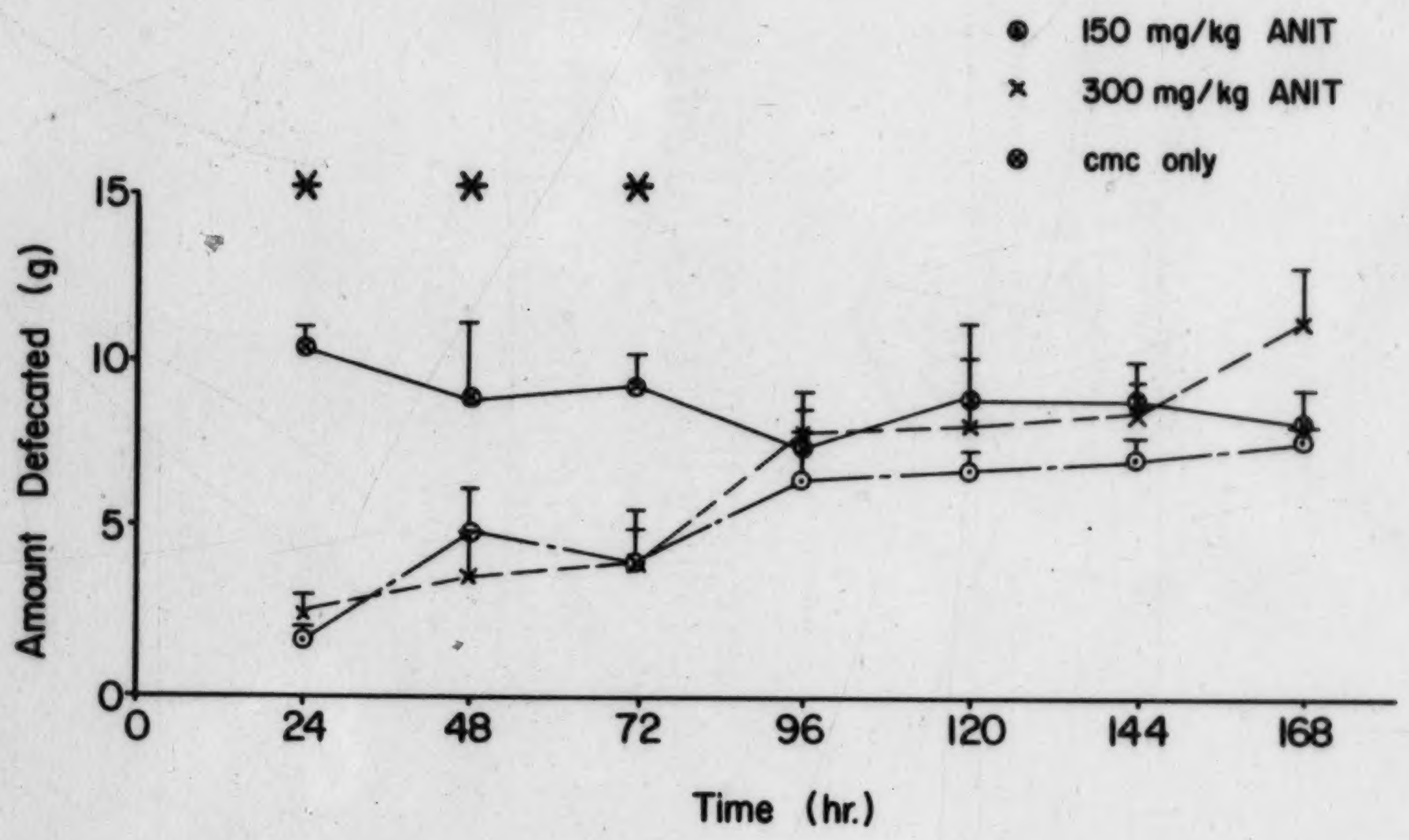


Figure 20. Weight (mean ± se) of feces excreted by rats given ANIT in peanut oil (n=4). * $p < 0.05$

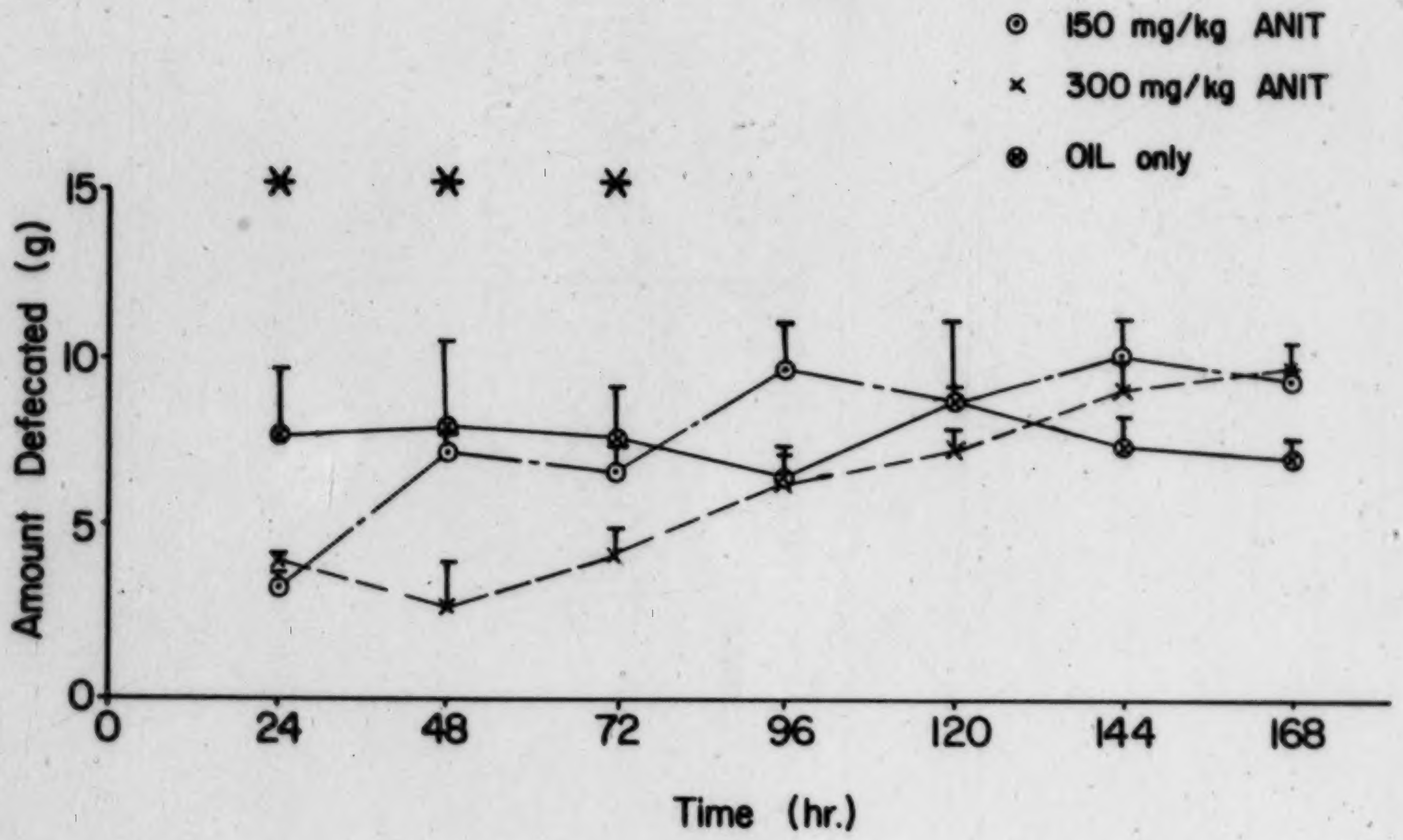


Table XI. Weight (Mean ± SE) of Feces Excreted by Rats Given ANIT in Peanut Oil and Carboxymethyl Cellulose (n=4). $^*p < 0.05$

ANIT IN OIL

Dose		Time (hr)							Total
(mg/kg ANIT)		24	48	72	96	120	144	168	Defecated (g)
150	Mean	3.32*	7.25	6.63	9.84	8.83	10.21	9.43	55.51
	SE	0.41	0.56	0.94	1.29	0.45	0.98	0.40	4.06
300	Mean	4.06*	2.70*	4.31*	6.40	7.40	9.33	9.76	43.96
	SE	0.23	1.40	0.77	0.88	0.52	0.75	0.68	3.08
Oil	Mean	7.81	8.03	7.76	6.57	8.76	7.46	7.14	53.53
	SE	2.04	2.46	1.48	0.86	2.37	0.97	0.52	8.85

ANIT IN CMC

Dose		Time (hr)							Total
(mg/kg ANIT)		24	48	72	96	120	144	168	Defecated (g)
150	Mean	1.68*	4.95*	4.01*	6.58	6.75	7.10	7.69	38.76
	SE	0.43	1.30	0.89	1.48	0.59	0.59	0.60	3.48
300	Mean	2.53*	3.60*	4.01*	7.94	8.22	8.40	11.36	46.06
	SE	0.64	1.33	1.57	1.17	1.89	1.66	1.65	6.60
CMC	Mean	10.39	8.96	9.29	7.58	8.95	8.87	8.22	62.26
	SE	0.54	2.31	0.98	1.00	2.29	0.57	1.07	5.99

Figure 21. Urinary excretion rate (mean ± se) of gold after administration of ^{198}Au-ATM (1.0mg Au/kg, im) in rats given ANIT in carboxymethylcellulose 24 hr. beforehand (n=4).

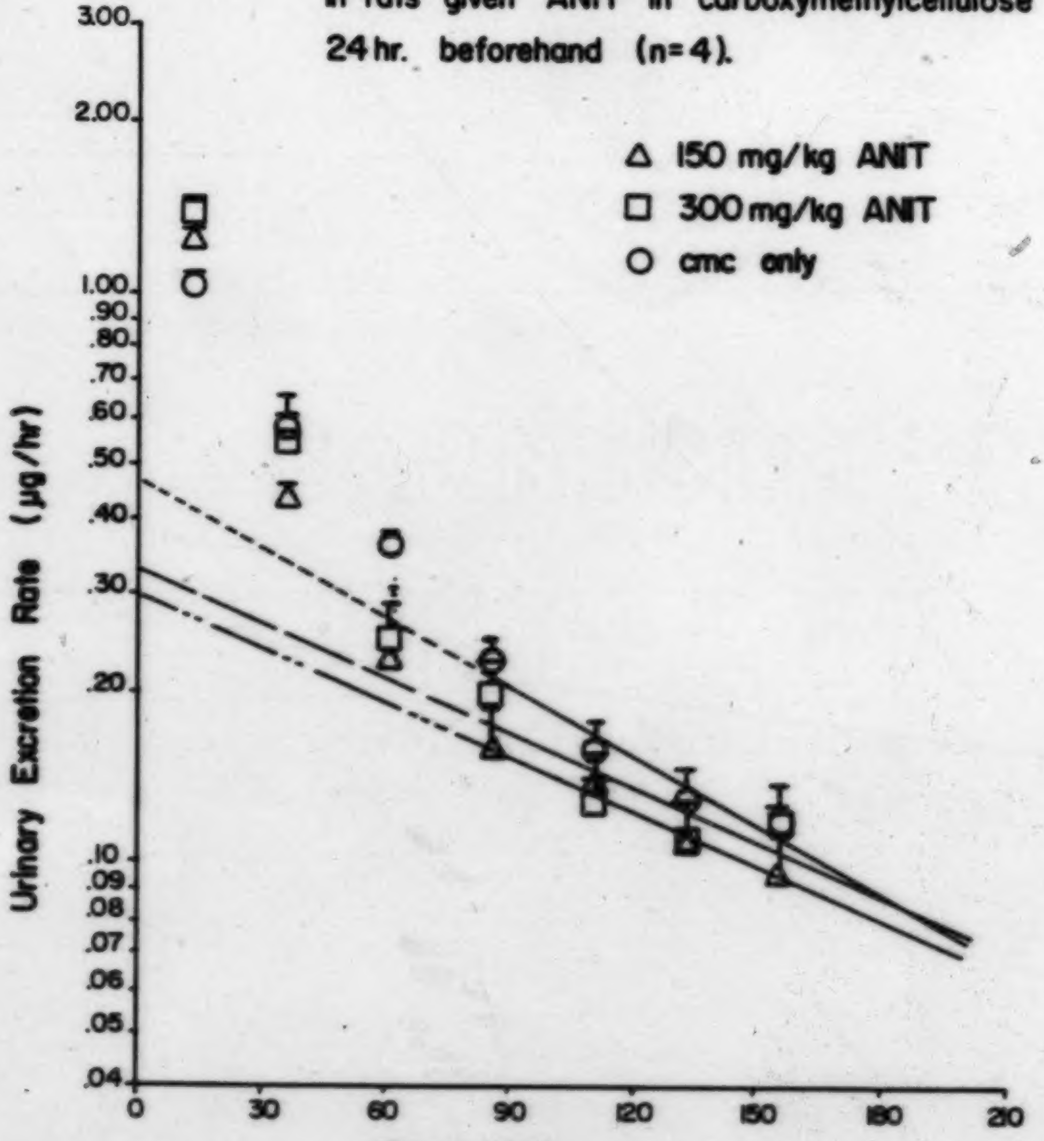


Figure 22. Urinary excretion rate (mean ± se) of gold after administration of ^{198}Au-ATM (1.0mg Au/kg, im) in rats given ANIT in peanut oil 24 hr. beforehand (n=4).

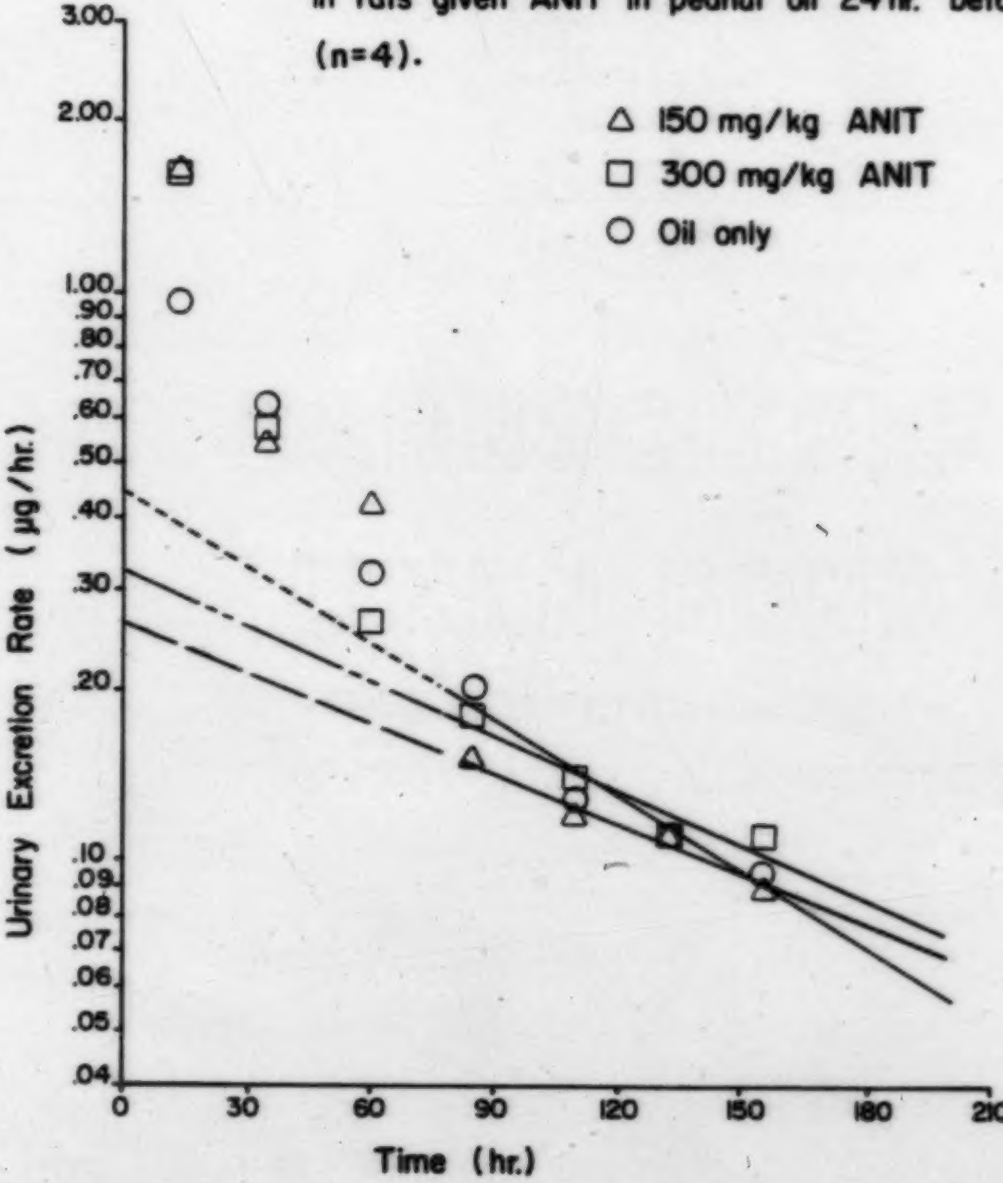


Figure 23. Fecal excretion rate (mean ± se) of gold after administration of ^{198}Au-ATM (1.0mg Au/kg,im) in rats given ANIT in carboxymethylcellulose 24 hr. beforehand (n=4).

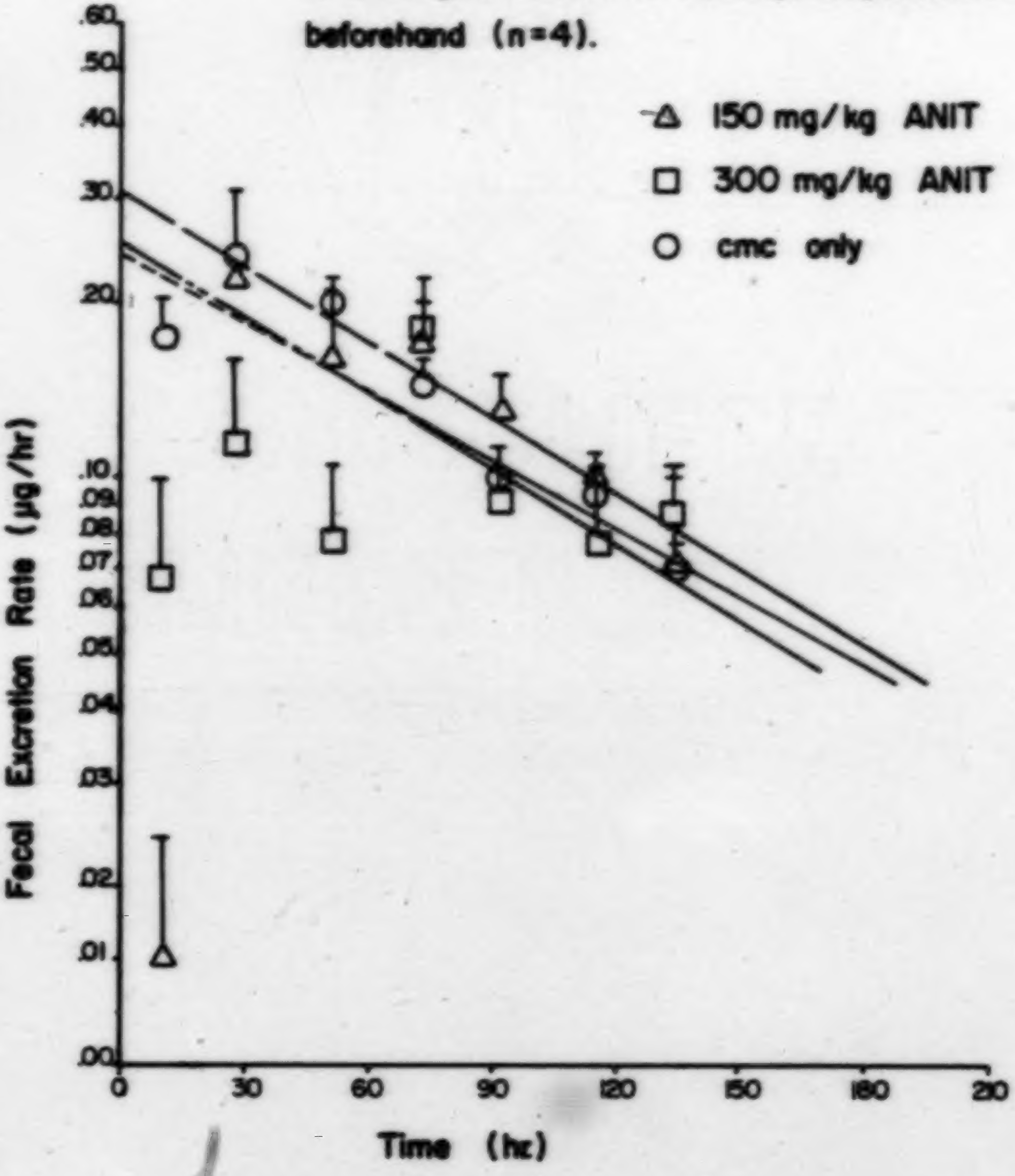
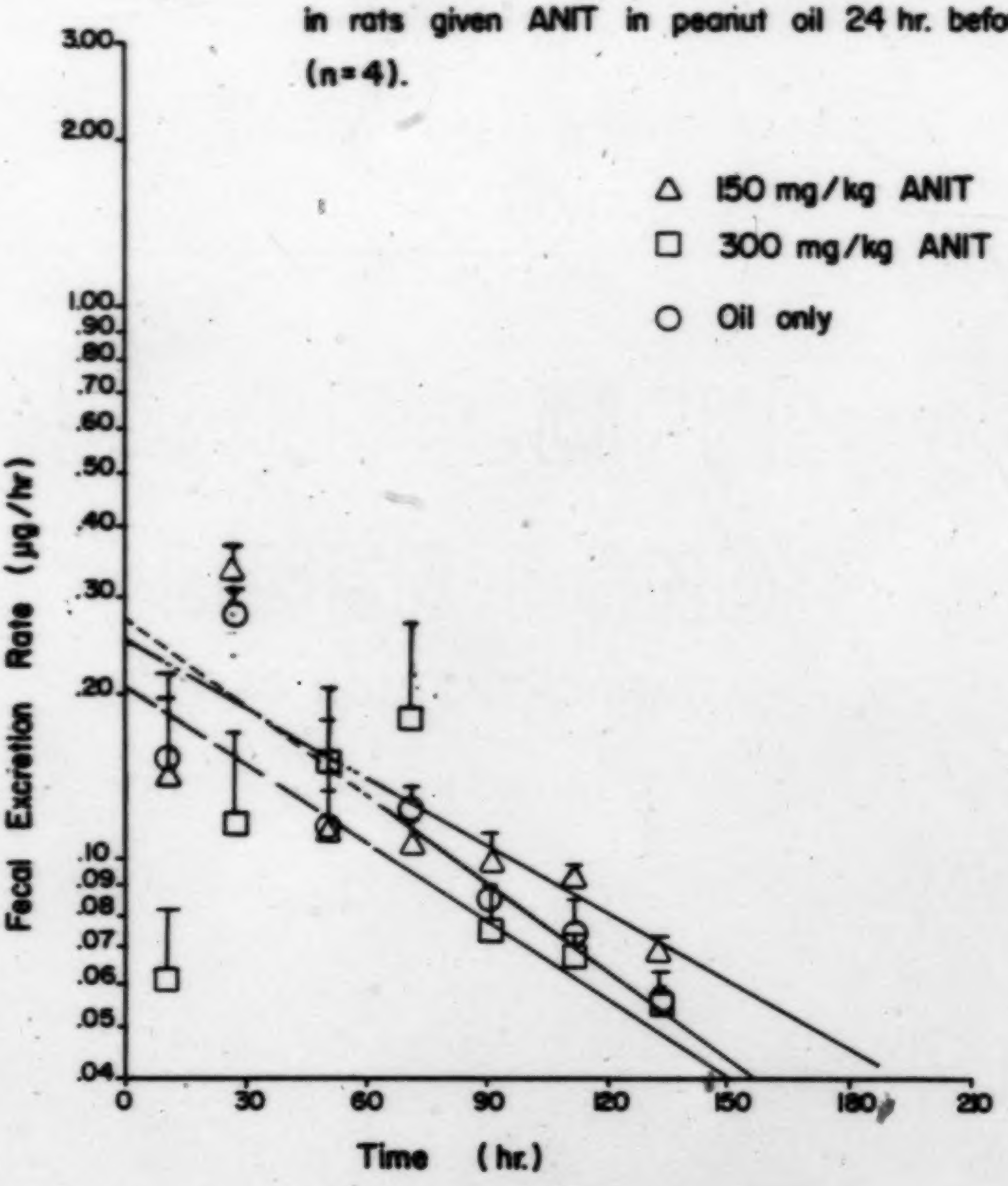


Figure 24. Fecal excretion rate (mean ± se) of gold after administration of ^{198}Au-ATM (1.0 mg Au/kg, im) in rats given ANIT in peanut oil 24 hr. beforehand (n=4).



were curvilinear. Mean values of K are summarized in Table XII. No significant differences were found between ANIT-treated and control rats.

Data from individual rats is tabulated in Appendix A (Tables A5-A14).

D. ISOLATION AND IDENTIFICATION OF GOLD METALLOTHIONEIN

Figure 25 shows a typical elution profile of rat liver supernatant after a single im injection of ATM (1.0 mg Au/kg). Peak I eluted at the column void volume and was found to contain protein(s) with a MW greater than or equal to 70,000 daltons (the exclusion limit of Sephadex G-75). Peak II, which was not completely separated from Peak I, had an elution volume similar to chymotrypsinogen A suggesting protein(s) with a MW of 25,000 daltons. However, a reduced absorbance at 280 nm was observed for Peak II. A protein, such as MT, that contains few or no aromatic amino acids could account for this reduction in absorbance. The last fraction eluted from the column (Peak III) contained l.m.w. amino acid fragments and the added mercaptoethanol. Using Bradford's microprotein assay, Peaks I, II and III had protein concentrations of 0.133, 0.064 and 0.004 mg/ml, respectively. Flameless AAS was too insensitive to measure gold concentration but NAA showed that all peaks contained gold. The exceptionally high concentration of gold in Peak III could be due to generalized binding to the protein fragments or to displacement of gold from proteins in the first two peaks by the isolation technique. A similar gel filtration pattern (Figure 26) was observed for rat liver supernatant after 14 weekly injections of ATM (1.0 mg Au/kg,im). Three peaks were separated and correspond to h.m.w.

Table XII. Mean Apparent Elimination Rate Constants (K) of Gold (^{198}Au-ATM) in Rats Given ANIT in Peanut Oil and Carboxymethyl Cellulose (n=4). * $p < 0.05$

ANIT IN OIL

Dose (mg/kg ANIT)		From Urinary Data K (hr^{-1})	From Fecal Data K (hr^{-1})
150	Mean	0.00747	0.00602
	SE	0.00062	0.00166
300	Mean	0.00831	0.00773
	SE	0.00122	0.00250
Oil	Mean	0.00891	0.00955
	SE	0.00111	0.00142

ANIT IN CMC

Dose (mg/kg ANIT)		K (hr^{-1})	K (hr^{-1})
150	Mean	0.00748	0.00753
	SE	0.00123	0.00233
300	Mean	0.00833	0.00983
	SE	0.00126	0.00124
CMC	Mean	0.00865	0.00835
	SE	0.00116	0.00087

Figure 25. Typical Sephadex G-75 elution profile of rat-liver supernatant after a single injection of ATM (1.0 mg Au/kg,im).

Liver supernatant (10 ml) was applied to a Sephadex G-75 column (2.6 x 90 cm) and 5 ml fractions were collected. (- - -) absorbance at 250 nm and (——) absorbance at 280 nm.

Peaks	I	II	III
Fractions	32-39	40-65	75-115
Volume (ml)	40	130	205
Protein (mg/ml)	0.133	0.064	0.004
Gold (μg/ml)	0.059	0.019	0.159

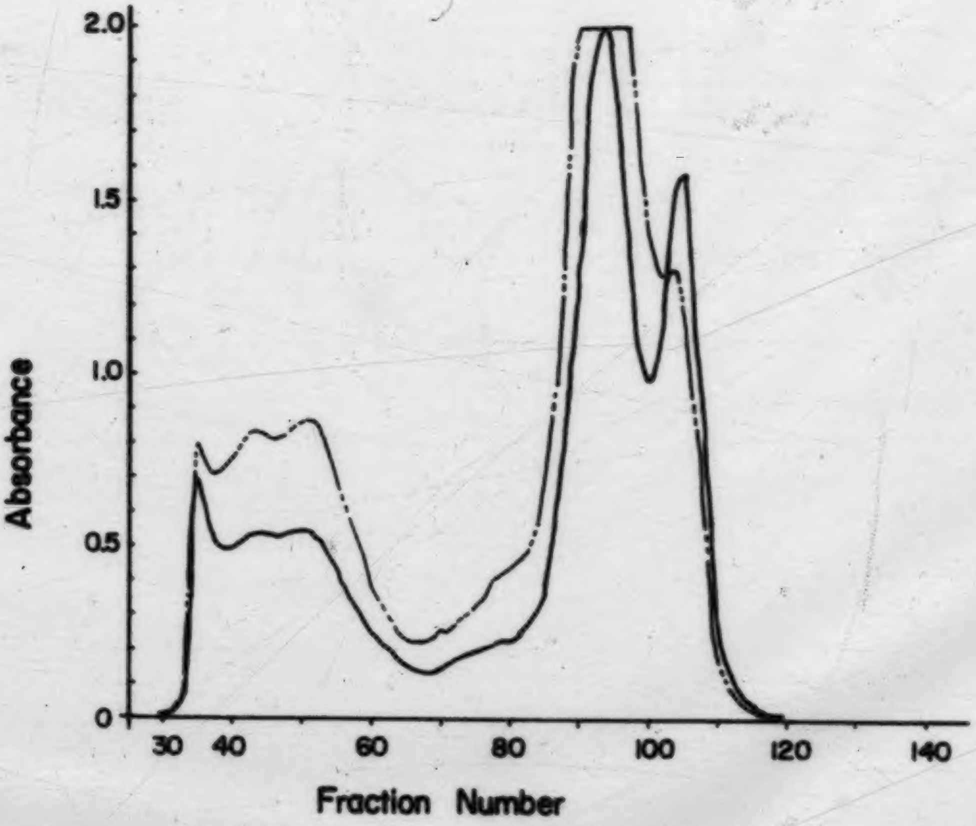
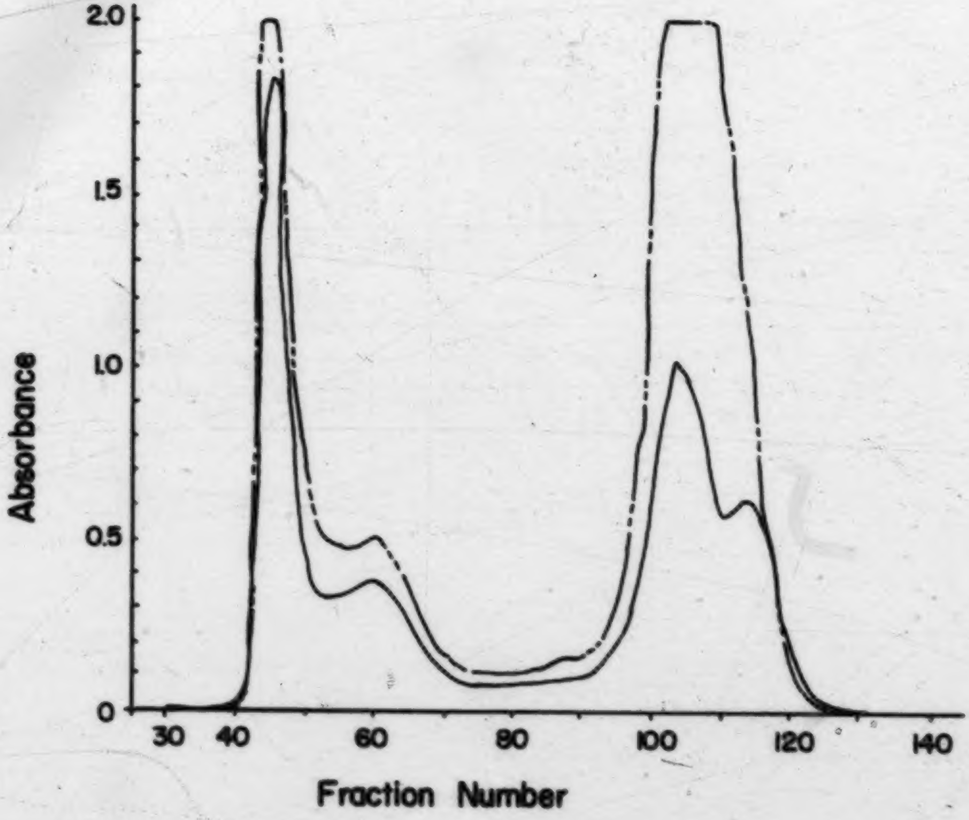


Figure 26. Typical Sephadex G-75 elution profile of rat-liver supernatant after 14 injections of ATM (1.0 mg Au/kg/week,im).

Liver supernatant (10 ml) was applied to a Sephadex G-75 column (2.6 x 90 cm) and 5 ml fractions were collected. (-·-) absorbance at 250 nm and (—) absorbance at 280 nm.

Peaks	I	II	III
Fractions	40-51	52-74	91-124
Volume (ml)	60	115	170
Protein (mg/ml)	0.069	0.075	0.000
Gold (μg/ml)	0.063	0.024	0.044



proteins (Peak I), l.m.w. proteins including MT Peak (II) and l.m.w. peptides (Peak III). Although more total gold was administered, protein and gold concentrations were similar to those measured after a single injection of ATM (Figure 25). These data indicate that other tissues must store much of the gold (ATM) administered over a prolonged period because the concentration of soluble proteins in the liver did not increase in response to the increased amount of gold in the body.

Figure 27 shows a typical elution profile of rat kidney supernatant after a single injection of ATM (1.0 mg Au/kg,im). The h.m.w. protein Peak (I) and the l.m.w. protein Peak II were barely discernable above background. Since less kidney supernatant was applied to the column the peak generally contained less protein than did the corresponding peaks of the rat liver supernatant after a single injection of ATM (Figure 25). However, significantly more gold was measured in each of the kidney supernatant peaks indicating that the soluble proteins of the kidney may have a greater affinity for gold (ATM) than those in the liver. Column chromatography of rat kidney supernatant after repeated administration of ATM (14 weekly injections) again gave 3 distinct peaks (Figure 28). These peaks contained similar amounts of protein but significantly less gold than after a single ATM injection (Figure 27).

Figure 27. Typical Sephadex G-75 elution profile of rat-kidney supernatant after a single injection of ATM (1.0 mg Au/kg,im).

Kidney supernatant (5 ml) was applied to a sephadex G-75 column (1.6 x 90 cm) and 2.5 ml fractions were collected. (-··-) absorbance at 250 nm and (——) absorbance at 280 nm.

Peaks	I	II	III
Fractions	24-29	30-50	55-85
Volume (ml)	15	52.5	77.5
Protein (mg/ml)	0.102	0.057	0.007
Gold (µg/ml)	0.425	0.351	0.439

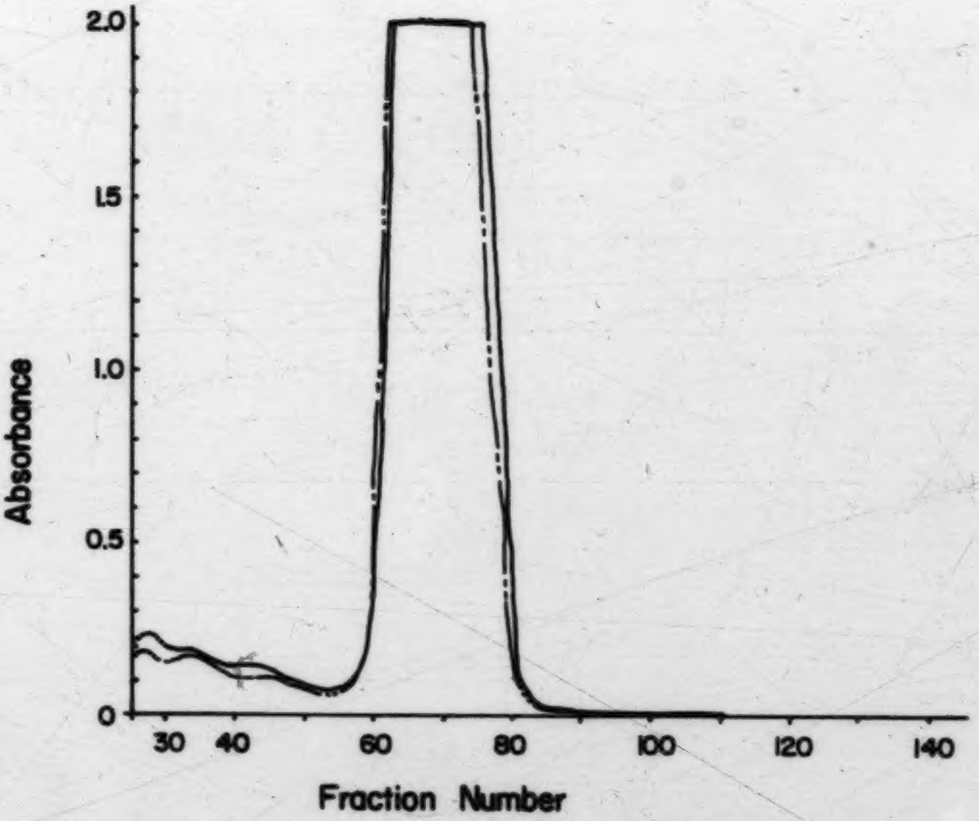
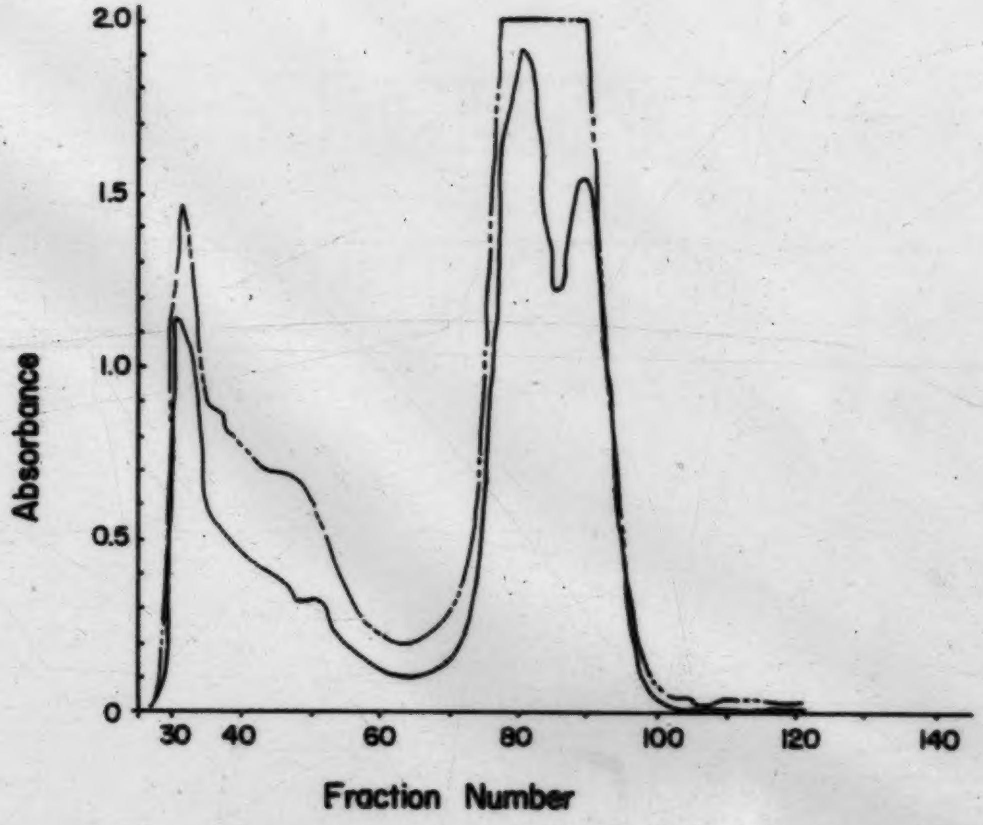


Figure 28. Typical Sephadex G-75 elution profile of rat-kidney supernatant after 14 injections of ATM (1.0 mg Au/kg/week,im).

Kidney supernatant (5 ml) was applied to a Sephadex G-75 column (1.6 x 90 cm) and 2.5 ml fractions were collected. (-··-) absorbance at 250 nm and (—) absorbance at 280 nm.

Peak	I	II	III
Fractions	27-36	37-60	66-100
Volume (ml)	25	60	87.5
Protein (mg/ml)	0.100	0.041	0.010
Gold (µg/ml)	0.180	0.135	0.194



DISCUSSION

Rats excreted gold in both urine and feces after injections of sodium aurothiomalate,im. The urinary and fecal excretion patterns were similar to those reported for RA patients treated with various soluble gold compounds (Freyberg, Block _et al._, 1941; 1942; Smith, Peak _et al._, 1958; Sliwinski, Zvaifler & Rubin, 1966; Mascarenhas, Granda & Freyberg, 1972; Harth, 1974; Leroy, Bureau _et al._, 1978).

A. ACUTE STUDIES

After single injections of ATM (0.5 mg and 1.0 mg Au/kg,im) urinary gold excretion peaked within 24 hr and fecal excretion reached a maximum by day 2. The gold concentrations in urine and feces then declined over the next week. After single doses, total amounts of gold recovered in urine and feces over 7 days were dose-related. However, this was not always obvious because of inter-animal variation. Mean values for K obtained from both urinary and fecal data were similar for single-dose studies.

B. CHRONIC STUDIES

After 13 weekly injections of ATM (1.0 mg Au/kg,im) rats given 1.0 mg ^{198}Au-ATM excreted amounts of ^{198}Au in urine and feces similar to those found in the 1.0 mg — acute studies. However, mean values of K, determined from urinary and fecal data were significantly increased in agreement with Walz, Griswold _et al._ (1980). This indicates that during multiple dosing, more of the current dose of gold is excreted in a given time, in comparison with previous doses. It is important to recognize that only ^{198}Au in urine and feces was measured, not _total_ gold; thus ^{198}Au excretion will represent only a portion of the total gold excreted in urine and feces and result in an apparent increase in K.

It is evident from the curvilinear nature of the excretion-time plots that equations describing the excretion process must contain at least two exponentials. However, the variance of the data did not allow definition of more than one of these exponentials (i.e. K). In hindsight, the experiments could have been improved by also measuring blood or serum gold concentrations after ^{198}Au iv and im, as well as urinary and fecal concentrations. Determination of the kinetics of gold disposition in more detail would then have been possible, in particular, determination of urinary (K_u) and fecal (K_f) excretion rate constants. The data only allowed me to use the intercept of the terminal excretory phases to estimate the ratio between K_u and K_f. Thus in each rat, the intercept of the urinary or fecal excretion/time plot at time zero was obtained. Assuming that the same processes of distribution and absorption apply in each animal for both urinary and fecal excretion, the ratio of the two intercepts should provide an indication of the difference between K_u and K_f. Using this technique, K_u/K_f was 1.51 ± 0.07. It must be stressed that this intercept is a composite of several functions (Gibaldi & Perrier, 1975) and may not represent the 'true' value of the ratio.

The experiments could also have been improved by extending the observation time. As indicated earlier, the terminal half-life of gold may be > 20 days (Mason et al., 1977c; 1979; 1981). If gold does undergo the triphasic excretion process described by others, then my experiments would only have detected the first and second phases, and the 'terminal' half-life observed is not the true terminal half-life, but the half-life of the 'β' phase. The problem inherent in my experiments is the use of the readily accessible gold isotope ^{198}Au. Its half-life is too short to permit meaningful experiments of longer than 10 days duration. Others (Gerber, Paulus et al., 1972a; 1974; Gottlieb, Smith & Smith, 1974; Mason et al., 1977c; 1979; 1981) have used ^{195}Au which can be followed for > 6 months because of its long half-life. However, ^{195}Au decays to ^{195}Pt and thus as the experiment progresses, results could be influenced by the presence of ^{195}Pt. Some authors have noted minor differences in elimination rate constants when ^{195}Au, compared to ^{197}Au, is used (Gottlieb, Smith & Smith, 1974).

Inter-animal variation is always a problem in experiments in which urinary and fecal excretion of a drug are measured. My estimates of K should not be affected by the sampling time since this was sufficiently frequent to reduce this type of variation to insignificant levels when one considers the value of K (Gibaldi & Perrier, 1975). However, urine and feces do show diurnal excretion patterns. This potential problem was overcome by starting each experiment at the same time of the day. The inter-animal variation observed in these experiments is similar to that observed by others in studies of gold excretion in both animals and man (Gottlieb, 1977). Thus, my estimates of K, though obviously estimates are within the range reported by others (Gerber, Paulus et al., 1972a; Harth, 1974; Walz, Griswold et al., 1980).

C. EXPERIMENTS WITH ANIT

Although our results indicate urine as the primary route of elimination of soluble gold complexes, a significant amount, about 6 - 8% of the dose, is excreted in the feces. Biliary secretion into the gastrointestinal tract could contribute to this. Several investigators have examined the biliary excretion of heavy metals such as mercury (Norseth & Clarkson, 1971; Cherian & Vostal, 1973), lead (Klaasen & Shoeman, 1974), and cadmium (Cikrt & Tichy, 1974), but few have studied gold. When T-tube drainage of the bile duct was instituted in a patient being treated with ATM, less than half the amount of gold formerly excreted in the feces was recovered from bile (Kapelowitz, Nelp et al., 1964). In a more recent study in rats, bile duct cannulations performed before the administration of auranofin showed that enterohepatic circulation was not the cause of the elevated serum and blood gold levels observed 24-48 hrs post-administration (Walz, Griswold et al., 1980). No similar studies however have been reported for ATM or other injectable gold compounds. ANIT is a cholestatic agent. Doses in excess of 150 mg/kg induce biliary stasis, complete within 24 hr and lasting 3 to 5 days (Plaa & Priestly, 1976). If gold were excreted via the bile, ANIT would be expected to reduce this until bile flow returned to normal, and, during biliary stasis, more gold might be excreted in the urine to compensate for this reduction.

In the first 72 hr, significantly more gold was recovered from urine, and significantly less from feces, of ANIT-treated rats than controls. This initial reduction in fecal gold content appeared to correspond to a reduction in the amount of feces excreted by treated rats, and the greater excretion of gold in the urine at this time may have been compensatory. After 72 hr, the amount of gold in the feces was similar for both treated and control rats. Thus, the amount of gold excreted in the feces appears to be related to the amount of feces produced and the biliary tract may participate in the transport of gold into the gastrointestinal tract. The relative contributions of biliary secretion and transport were not measured because bile duct cannulations were not performed.

In animals pretreated with ANIT, mean values of K were not significantly different from controls.

D. ISOLATION AND IDENTIFICATION OF GOLD METALLOTHIONEIN

Since the discovery and characterization of MT, this unique l.m.w. cytoplasmic metal-binding protein has received much attention. In various animal tissues its synthesis has been induced (at the transcriptional level) in response to bivalent metals such as cadmium, mercury, silver, bismuth and zinc (Kagi & Nordberg, 1978; Foulkes, 1982). Glucocorticoid hormones have also been shown to regulate MT synthesis (Etzel & Cousins, 1981). These observations suggest that MT has an important although as yet undefined biological function. The most widely accepted proposal is that MT functions both in regulating the metabolism and homeostasis of the essential metals and as a detoxifying agent by sequestering toxic metals (Kagi, 1980; Whanger, Oh & Deagen, 1981).

Gold(III) has been shown to elevate the amount of MT in rat-kidney (Mogilnicka & Piotrowski, 1977; 1979) and liver (Winge, Premakumar & Rajagopalan, 1978) but MT-induction in response to gold(I) has not yet been demonstrated (Turkall, Bianchine & Leber, 1977; Thompson, Blaszak et al., 1978). If small amounts of gold(I) were capable of inducing MT synthesis, this might explain the reduced toxicity observed in some patients receiving low doses of ATM/ATG at the beginning of treatment (Lawrence, 1976). Another possibility is that protection against chronic toxicity of gold might be conferred by pre-treatment with MT-synthesizing agents such as zinc (Mason, Sharma & McQueen, 1981).

After exposure to ATM, fractionation of liver and kidney supernatants by gel permeation chromatography resulted in the separation of three peaks as has been reported by others (Lawson, Danpure & Fyfe, 1977; Thompson, Blaszak et al., 1978). The first fraction, eluting at the column void volume, corresponded to h.m.w. protein(s) (MW > 70,000 daltons). Chromatographic behavior of the second fraction indicated the presence of a protein with a MW of about 25,000 daltons. This fraction was also thought to contain MT since absorbance at 280 nm was reduced indicating a protein with few or no aromatic amino acids. However, to confirm the presence of MT in Peak II, extensive purification of this fraction would be required before conclusive tests such as measurement of cysteine content, amino acid analysis, and gel electrophoresis, could be performed. In these preliminary studies unequivocal identification of this fraction as MT was not possible. Peak III, the last fraction eluted from the column, was found to contain predominantly amino acid fragments.

Following administration of a single dose of ATM, Peak I (for both liver and kidney supernatant) contained approximately twice as much protein as Peak II, and Peak III contained almost none. However, in all three kidney supernatant fractions, gold concentration was considerably higher than in the corresponding fractions of the liver supernatant.

These data suggest that kidney tissue has a much greater affinity for gold than liver, and confirms results (unpublished) that we observed when autopsies were performed on rats 72-120 hrs after administration of ANIT and ^{198}Au-ATM. After 14 weekly injections of ATM, protein and gold concentrations for all fractions of liver and kidney supernatant were not increased. Thus, administration of gold for prolonged periods does not appear to induce the synthesis of MT-like proteins in the liver or kidney, even though these organs have been reported to concentrate gold (Block, Buchanan & Freyberg, 1941; 1942; Swartz, Christian & Andrews, 1960; McQueen & Dykes, 1969; Gottlieb, Smith & Smith, 1972a; Kamel, Brown _et_ _al._, 1978b; Mason & Kingsford, 1979).

In summary, these investigations have shown:

1. That the excretion of ATM in urine and feces is dose-dependent, the amount excreted being directly proportional to the dose administered.
2. That repeated administration of ATM may induce an "apparent" increase in K.
3. That ANIT reduces fecal excretion of gold in the first 72 hrs probably because of a constipating effect. A compensatory increase in urinary gold excretion is observed during this time period. After 72 hrs, defecation and fecal gold elimination returns to normal.
4. That biliary secretion of gold may account in part for the excretion of gold in feces, and
5. That a l.m.w. gold-binding protein, resembling MT, can be isolated from rat-kidney and liver cytosol.

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APPENDIX A

Table A-1

The Amount of Gold Excreted in Urine per 24 hr After Administration of ^{198}Au-ATM, im.

Dose (mg Au/kg)	Rat	Weight (g)	Time (hr)							Total
			24	48	72	96	120	144	168	Excreted (µg)
0.5	1	374	8.38	6.10	4.09	3.72	1.74	1.95	1.84	27.82
	2	470	8.81	11.21	5.31	3.76	2.21	1.73	1.99	35.02
	3	448	19.61	15.37	7.97	6.32	3.83	3.04	1.93	58.07
	4	550	18.43	16.27	6.56	4.02	3.07	2.40	2.43	53.18
Mean			13.81	12.24	5.98	4.46	2.71	2.28	2.05	43.52
SE			3.02	2.32	0.83	0.63	0.46	0.29	0.13	7.21
1.0	5	520	43.62	29.53	15.36	10.17	7.95	3.70	3.86	114.19
	6	480	45.03	29.75	18.59	9.44	7.07	4.77	5.58	120.23
	7	250	12.54	9.46	5.11	3.35	2.72	1.97	2.21	37.36
	8	360	39.27	24.93	13.17	8.74	4.45	5.03	3.79	99.34
Mean			35.11	23.42	13.06	7.93	5.55	3.87	3.86	92.78
SE			7.62	4.78	5.75	1.55	1.20	0.69	0.69	18.99
1.0 weekly	9	480	35.61	32.98	15.61	10.47	5.52	4.37	3.26	107.82
	10	490	47.80	26.57	11.95	8.12	6.51	4.32	2.92	108.19
	11	410	46.62	27.13	12.92	10.43	6.09	4.96	2.72	110.87
	12	390	38.66	35.16	12.92	9.43	5.50	4.78	3.12	109.57
Mean			42.17	30.46	13.35	9.61	5.91	4.61	3.01	109.11
SE			2.98	2.13	0.79	0.55	0.24	0.16	0.12	0.70

Table A-2

The Amount of Gold Excreted in Feces per 24 hr After Administration of ^{198}Au-ATM, im.

Dose (mg Au/kg)	Rat	Weight (g)	Time (hr) 24	48	72	96	120	144	168	Total Excreted (μg)
0.5	1	374	1.56	1.72	2.32	2.10	1.37	1.11	1.02	11.26
	2	470	0.37	1.94	2.39	1.64	1.68	1.27	0.92	10.21
	3	448	12.44	5.97	3.66	3.31	2.51	1.95	1.49	31.33
	4	550	2.52	3.25	4.06	2.05	1.75	1.00	1.05	15.68
Mean			4.22	3.22	3.11	2.28	1.83	1.33	1.12	17.12
SE			2.77	0.98	0.44	0.36	0.24	0.21	0.13	4.88
1.0	5	520	0.02	17.34	7.80	5.96	4.06	2.72	2.94	40.84
	6	480	1.28	9.85	7.75	4.30	4.41	2.98	2.10	32.67
	7	250	4.67	4.15	3.68	2.52	1.74	1.83	1.36	19.95
	8	360	0.72	10.96	4.17	5.66	3.92	3.36	3.26	32.05
Mean			1.67	10.58	5.85	4.61	3.53	2.72	2.42	31.38
SE			1.03	2.70	1.12	0.78	0.61	0.33	0.43	4.30
1.0 weekly	9	480	4.36	7.35	5.17	4.79	3.02	2.48	1.88	29.05
	10	490	1.54	10.07	6.52	4.84	2.99	3.01	1.69	30.66
	11	410	3.10	6.77	5.08	3.63	2.74	1.88	1.56	24.76
	12	390	4.39	9.00	4.73	5.50	2.64	2.61	1.69	30.56
Mean			3.35	8.30	5.38	4.69	2.85	2.50	1.71	28.76
SE			0.67	0.76	0.39	0.39	0.09	0.23	0.07	1.38

Table A-3

Urinary Excretion Rate (μg/hr) of Gold After Administration of ^{198}Au-ATM,im.

Dose (mg Au/kg)	Rat	Weight (g)	Mid-time of Each Collection Period (hr)						
			12	36	60	84	108	132	156
0.5	1	374	0.41	0.27	0.15	0.17	0.07	0.09	0.08
	2	470	0.48	0.48	0.22	0.14	0.09	0.08	0.09
	3	448	1.13	0.63	0.34	0.26	0.16	0.11	0.10
	4	550	0.80	0.65	0.26	0.17	0.14	0.10	0.10
Mean			0.71	0.51	0.24	0.19	0.11	0.10	0.09
SE			0.17	0.09	0.04	0.03	0.02	0.01	0.01
1.0	5	520	1.82	1.24	0.63	0.47	0.28	0.17	0.16
	6	480	1.88	1.29	0.75	0.44	0.25	0.21	0.24
	7	250	0.51	0.41	0.20	0.14	0.12	0.08	0.09
	8	360	1.63	1.04	0.59	0.34	0.19	0.21	0.16
Mean			1.46	1.00	0.54	0.35	0.27	0.17	0.16
SE			0.32	0.20	0.12	0.07	0.04	0.03	0.03
1.0 weekly	9	480	1.45	1.32	0.71	0.43	0.23	0.18	0.14
	10	490	1.95	1.06	0.54	0.33	0.28	0.17	0.13
	11	410	1.90	1.09	0.59	0.43	0.26	0.20	0.12
	12	390	1.58	1.41	0.59	0.38	0.23	0.19	0.13
Mean			1.72	1.22	0.61	0.39	0.25	0.19	0.13
SE			0.12	0.09	0.04	0.02	0.01	0.01	0.00

Table A-4

Fecal Excretion Rate (µg/hr) of Gold After Administration of ^{198}Au-ATM,im.

Dose (mg Au/kg)	Rat	Weight (g)	Mid-time of Each Collection Period (hr)						
			12	36	60	84	108	132	156
0.5	1	374	0.08	0.08	0.09	0.09	0.05	0.05	0.04
	2	470	0.02	0.08	0.10	0.06	0.07	0.06	0.04
	3	448	0.71	0.25	0.16	0.14	0.11	0.07	0.07
	4	550	0.11	0.13	0.16	0.09	0.08	0.04	0.04
Mean			0.23	0.14	0.13	0.10	0.08	0.06	0.05
SE			0.16	0.04	0.02	0.02	0.01	0.01	0.01
1.0	5	520	0.00	0.73	0.32	0.27	0.14	0.12	0.13
	6	480	0.05	0.43	0.31	0.20	0.15	0.13	0.09
	7	250	0.19	0.18	0.14	0.11	0.07	0.08	0.06
	8	360	0.03	0.46	0.19	0.22	0.17	0.14	0.13
Mean			0.07	0.45	0.24	0.20	0.13	0.12	0.10
SE			0.04	0.11	0.04	0.03	0.02	0.01	0.02
1.0 weekly	9	480	0.18	0.29	0.24	0.20	0.13	0.10	0.08
	10	490	0.06	0.40	0.30	0.20	0.13	0.12	0.07
	11	410	0.13	0.27	0.23	0.15	0.12	0.08	0.07
	12	390	0.18	0.36	0.22	0.22	0.11	0.11	0.07
Mean			0.14	0.33	0.25	0.19	0.12	0.10	0.07
SE			0.03	0.03	0.02	0.01	0.01	0.01	0.00

Table A-5

The Amount of Gold Excreted in Urine After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in Rats Given ANIT in Peanut Oil 24 hr Beforehand.

Dose (mg/kg ANIT)	Rat	Weight (g)	Time (hr)							Total
			24	48	72	96	120	144	168	Excreted (µg)
150	13	307	36.39	10.26	4.94	4.27	2.92	2.24	2.75	63.77
	14	296	37.64	14.98	6.35	4.82	3.84	2.87	2.70	73.20
	15	285	40.67	15.46	6.44	4.41	3.30	1.86	2.49	74.63
	16	303	39.15	14.80	6.35	4.90	3.72	2.79	2.55	74.26
Mean			38.46	13.88	6.02	4.60	3.45	2.44	2.62	71.47
SE			0.93	1.21	0.36	0.15	0.21	0.24	0.06	2.48
300	17	318	41.80	14.02	11.07	5.15	3.13	2.74	1.86	79.77
	18	292	38.82	13.07	6.30	3.65	2.56	3.18	2.43	70.01
	19	291	34.31	9.61	4.07	2.42	1.65	1.70	1.75	55.45
	20	314	39.75	12.61	9.05	4.90	3.12	2.86	3.00	75.29
Mean			38.67	12.33	7.61	4.03	2.62	2.62	2.26	70.13
SE			1.58	0.95	1.55	0.63	0.35	0.32	0.29	5.28
Oil	21	302	27.41	19.06	8.05	4.97	3.73	2.86	3.05	69.13
	22	281	18.67	12.69	5.93	3.55	2.91	2.26	1.86	47.87
	23	281	21.35	13.30	8.14	4.99	2.76	2.45	1.87	54.86
	24	288	21.55	14.45	8.74	6.55	3.32	2.70	2.05	59.36
Mean			22.25	14.88	7.72	5.02	3.18	2.57	2.21	57.81
SE			1.84	1.44	0.61	0.61	0.22	0.13	0.28	4.45

Table A-6

The Amount of Gold Excreted in Feces After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in Rats Given ANIT in Peanut Oil 24 hr Beforehand.

Dose (mg/kg ANIT)	Rat	Weight (g)	Time (hr)							Total
			24	48	72	96	120	144	168	Excreted (μg)
150	13	307	5.71	10.85	2.54	2.31	2.28	2.19	1.62	27.50
	14	296	0.42	9.26	1.59	2.20	3.09	2.32	1.85	20.73
	15	285	0.49	9.82	2.56	2.99	1.94	1.84	1.40	16.04
	16	303	7.25	7.77	4.10	3.42	2.75	2.23	1.94	29.46
Mean			3.47	8.18	2.70	2.73	2.52	2.15	1.70	23.43
SE			1.77	1.28	0.52	0.29	0.25	0.11	0.12	3.09
300	17	318	1.80	5.93	2.59	4.07	2.79	1.92	1.67	20.77
	18	292	0.63	3.71	2.69	1.75	1.60	1.64	1.10	13.12
	19	291	0.81	0.37	2.42	0.00	0.86	1.19	0.97	12.68
	20	314	2.67	1.02	7.78	11.36	1.10	1.56	1.79	27.28
Mean			1.48	2.76	3.87	4.30	1.61	1.58	1.38	18.46
SE			0.47	1.28	1.30	2.50	0.53	0.15	0.20	3.48
Oil	21	302	6.48	5.89	4.41	3.44	2.08	1.79	1.43	25.52
	22	281	1.47	8.99	2.88	2.15	1.86	1.20	1.37	19.92
	23	281	3.04	5.53	4.71	3.25	2.21	1.51	1.26	21.51
	24	288	3.70	6.54	3.92	3.91	1.98	2.44	1.46	23.95
Mean			3.67	6.74	3.98	3.19	2.03	1.74	1.38	22.73
SE			1.05	0.78	0.40	0.37	0.07	0.26	0.04	1.25

Table A-7

The Amount of Gold Excreted in Urine After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in Rats Given ANIT in Carboxymethyl Cellulose 24 hr Beforehand.

Dose (mg/kg ANIT)	Rat	Weight (g)	Time (hr)							Total
			24	48	72	96	120	144	168	Excreted (µg)
150	25	280	30.97	10.09	4.46	3.04	2.41	1.41	1.51	53.89
	26	297	27.74	12.31	5.53	3.59	3.10	2.27	2.25	56.79
	27	315	30.72	10.61	7.32	6.31	4.54	3.91	3.33	66.78
	28	259	25.95	9.22	4.77	3.07	3.61	2.63	2.24	51.49
Mean			28.85	10.56	5.52	4.00	3.42	2.56	2.34	57.24
SE			1.21	0.65	0.64	0.78	0.45	0.52	0.38	3.36
300	29	273	34.90	9.78	4.41	3.38	2.97	1.53	1.99	58.96
	30	266	34.25	11.69	4.72	4.47	2.98	3.21	2.64	63.96
	31	387	20.33	16.05	8.03	7.57	3.53	2.36	3.67	61.54
	32	277	35.19	15.50	5.89	5.84	3.64	1.63	3.15	70.84
Mean			31.17	13.26	5.76	5.32	3.28	2.18	2.86	63.83
SE			3.62	1.51	0.82	0.90	0.18	0.39	0.36	2.55
CMC	33	288	21.55	14.43	8.74	6.55	3.32	2.70	2.05	59.36
	34	410	25.92	9.42	10.57	7.09	5.27	3.96	3.72	65.95
	35	302	27.41	19.06	8.05	4.97	3.73	2.86	3.05	69.13
	36	281	21.35	13.30	8.14	4.99	2.76	2.45	1.87	54.86
Mean			24.06	14.06	8.88	5.90	3.77	2.99	2.67	62.33
SE			1.54	1.98	0.59	0.54	0.54	0.33	0.44	3.21

Table A-8

The Amount of Gold Excreted in Feces After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in Rats Given ANIT in Carboxymethyl Cellulose 24 hr Beforehand.

Dose (mg/kg ANIT)	Rat	Weight (g)	Time (hr)							Total
			24	48	72	96	120	144	168	Excreted (μg)
150	25	280	0.74	2.08	1.55	2.63	1.97	1.68	1.38	12.03
	26	297	0.06	1.79	7.21	5.42	2.92	2.34	1.54	21.28
	27	315	0.63	8.13	4.42	5.51	3.81	2.39	2.07	26.96
	28	259	0.03	9.05	2.40	4.21	3.51	2.73	1.69	23.63
Mean			0.37	5.26	3.90	4.44	3.05	2.29	1.67	20.97
SE			0.19	1.93	1.26	0.67	0.41	0.22	0.15	3.20
300	29	273	3.23	4.46	2.19	2.06	2.58	1.24	1.38	17.14
	30	266	0.65	4.14	1.76	2.73	2.90	2.29	1.57	16.04
	31	387	0.04	0.00	0.00	12.46	1.92	1.70	3.50	19.62
	32	277	2.27	2.50	3.17	2.39	2.12	1.00	1.77	15.22
Mean			1.55	2.78	1.78	4.91	2.38	1.56	2.06	17.01
SE			0.73	1.02	0.66	2.52	0.32	0.28	0.49	0.96
CMC	33	288	3.70	6.54	3.92	3.91	1.98	2.44	1.46	23.95
	34	410	3.01	4.79	6.47	4.61	3.20	2.79	2.44	27.31
	35	302	6.48	5.89	4.41	3.44	2.08	1.79	1.43	25.52
	36	281	3.04	5.53	4.71	3.25	2.21	1.51	1.26	21.51
Mean			4.06	5.69	4.88	3.80	2.37	2.13	1.65	24.57
SE			0.82	0.36	0.56	0.30	0.28	0.29	0.27	1.23

Table A-9

Weight of Feces Excreted by Rats Given ANIT in Peanut Oil.

Dose (mg/kg ANIT)	Rat	Weight (g)	Time (hr)							Total
			24	48	72	96	120	144	168	Defecated (g)
150	13	307	3.59	5.59	6.08	9.29	8.26	9.77	9.30	51.88
	14	296	2.24	7.81	5.68	7.07	7.86	8.28	8.52	47.46
	15	285	3.24	8.07	9.41	13.32	9.64	9.83	9.52	66.41
	16	303	4.20	7.52	5.35	9.69	9.57	12.96	10.44	56.29
Mean			3.32	7.25	6.63	9.84	8.83	10.21	9.45	55.51
SE			0.47	0.56	0.94	1.29	0.45	0.98	0.40	4.06
300	17	318	4.40	5.14	3.00	8.15	8.42	9.28	9.31	47.70
	18	292	3.94	5.11	6.48	7.34	8.00	9.60	9.82	50.29
	19	291	4.45	0.31	4.25	5.97	6.08	7.39	8.33	36.78
	20	314	3.45	0.25	3.50	4.15	7.08	11.06	11.86	41.05
Mean			4.06	2.70	4.31	6.40	7.40	9.33	9.76	43.96
SE			0.23	1.40	0.77	0.88	0.52	0.75	0.68	3.08
Oil	21	302	9.75	8.04	10.44	4.90	8.94	8.81	8.63	59.51
	22	381	1.68	4.75	4.25	5.45	5.57	4.68	6.76	33.14
	23	281	9.82	14.99	9.99	8.62	15.39	8.82	6.99	74.62
	24	288	9.99	4.34	6.36	7.30	5.15	7.53	6.19	46.86
Mean			7.81	8.03	7.76	6.57	8.76	7.46	7.14	53.53
SE			2.04	2.46	1.48	0.86	2.37	0.97	0.52	8.85

Table A-10

Weight of Feces Excreted by Rats Given ANIT in Carboxymethyl Cellulose.

Dose (mg/kg ANIT)	Rat	Weight (g)	Time (hr) 24	48	72	96	120	144	168	Total Defecated (g)
150	25	280	2.63	6.17	6.51	7.56	6.74	6.75	8.51	44.87
	26	297	0.56	1.68	2.37	3.83	5.32	8.79	8.89	31.44
	27	315	1.87	7.73	3.94	10.30	8.22	6.03	6.44	44.53
	28	259	1.66	4.23	3.22	4.62	6.72	6.83	6.92	34.20
Mean			1.68	4.95	4.01	6.58	6.75	7.10	7.69	38.76
SE			0.43	1.30	0.89	1.48	0.59	0.59	0.60	3.48
300	29	273	4.12	3.43	5.36	10.79	12.32	12.34	14.46	62.82
	30	266	2.48	6.28	3.30	5.50	6.85	7.59	7.60	39.60
	31	387	0.99	0.00	0.00	8.79	3.66	9.29	9.61	32.34
	32	277	2.52	4.68	7.38	6.69	10.05	4.39	13.75	49.46
Mean			2.53	3.60	4.01	7.94	8.22	8.40	11.36	46.06
SE			0.64	1.33	1.57	1.17	1.89	1.66	1.65	6.60
CMC	33	288	9.99	4.34	6.36	7.30	5.15	7.53	6.19	46.86
	34	410	12.00	8.48	10.36	9.50	6.32	10.33	11.06	68.05
	35	302	9.75	8.04	10.44	4.90	8.94	8.81	8.63	59.51
	36	281	9.82	14.99	9.99	8.62	15.39	8.82	6.99	74.62
Mean			10.39	8.96	9.29	7.58	8.95	8.87	8.22	62.26
SE			0.54	2.21	0.98	1.00	2.29	0.57	1.07	5.99

Table A-11

Urinary Excretion Rate (µg/hr) of Gold After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in Rats Given ANIT in Peanut Oil 24 hr Beforehand.

Dose (mg/kg ANIT)	Rat	Weight (g)	Mid-time of Each Collection Period (hr)						
			12	36	60	84	108	132	156
150	13	307	1.53	0.42	0.21	0.17	0.12	0.10	0.11
	14	296	1.58	0.61	0.27	0.19	0.16	0.13	0.11
	15	285	1.71	0.63	0.27	0.17	0.14	0.08	0.10
	16	303	1.64	0.60	0.27	0.19	0.15	0.13	0.11
Mean			1.62	0.57	0.26	0.18	0.14	0.11	0.11
SE			0.04	0.05	0.02	0.01	0.01	0.01	0.01
300	17	318	1.78	0.60	0.45	0.19	0.14	0.12	0.08
	18	292	1.65	0.56	0.25	0.14	0.11	0.14	0.10
	19	291	1.46	0.41	0.16	0.09	0.07	0.07	0.07
	20	314	1.69	0.54	0.36	0.18	0.14	0.12	0.12
Mean			1.65	0.53	0.42	0.15	0.12	0.11	0.09
SE			0.07	0.04	0.08	0.02	0.02	0.01	0.01
Oil	21	302	1.15	0.78	0.34	0.20	0.15	0.13	0.13
	22	281	0.79	0.52	0.25	0.15	0.12	0.10	0.08
	23	281	0.91	0.57	0.33	0.19	0.12	0.11	0.08
	24	288	0.94	0.60	0.36	0.25	0.14	0.11	0.09
Mean			0.95	0.62	0.32	0.20	0.13	0.11	0.10
SE			0.07	0.06	0.02	0.02	0.01	0.01	0.01

Table A-12

Fecal Excretion Rate (μg/hr) of Gold After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in Rats Given ANIT in Peanut Oil 24 hr Beforehand.

Dose (mg/kg ANIT)	Rat	Weight (g)	Mid-time of Each Collection Period (hr)						
			12	36	60	84	108	132	156
150	13	307	0.24	0.44	0.11	0.09	0.09	0.10	0.07
	14	296	0.02	0.38	0.07	0.09	0.13	0.10	0.08
	15	285	0.02	0.20	0.11	0.12	0.08	0.08	0.06
	16	303	0.30	0.32	0.17	0.14	0.11	0.10	0.08
Mean			0.15	0.34	0.12	0.11	0.10	0.10	0.07
SE			0.07	0.05	0.02	0.01	0.01	0.01	0.01
300	17	318	0.08	0.25	0.10	0.15	0.12	0.08	0.07
	18	292	0.03	0.16	0.11	0.07	0.07	0.07	0.05
	19	291	0.03	0.02	0.10	0.10	0.07	0.05	0.04
	20	314	0.11	0.04	0.31	0.43	0.05	0.07	0.07
Mean			0.06	0.12	0.16	0.19	0.08	0.07	0.06
SE			0.02	0.05	0.05	0.08	0.02	0.01	0.01
Oil	21	302	0.27	0.24	0.19	0.14	0.09	0.08	0.06
	22	281	0.06	0.37	0.12	0.09	0.07	0.05	0.06
	23	281	0.13	0.24	0.19	0.12	0.10	0.07	0.05
	24	288	0.16	0.27	0.16	0.15	0.09	0.10	0.07
Mean			0.16	0.28	0.17	0.12	0.09	0.08	0.06
SE			0.04	0.03	0.02	0.01	0.01	0.01	0.00

Table A-13

Urinary Excretion Rate (μg/hr) of Gold After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in Rats Given ANIT in Carboxymethyl Cellulose 24 hr Beforehand.

Dose (mg/kg ANIT	Rat	Weight (g)	Mid-time of Each Collection Period (hr)						
			12	36	60	84	108	132	156
150	25	280	1.35	0.42	0.18	0.12	0.10	0.06	0.06
	26	297	1.21	0.52	0.23	0.14	0.13	0.10	0.10
	27	315	1.34	0.44	0.30	0.24	0.19	0.17	0.14
	28	259	1.13	0.39	0.19	0.12	0.15	0.12	0.09
Mean			1.26	0.44	0.23	0.16	0.14	0.11	0.10
SE			0.05	0.03	0.03	0.03	0.02	0.02	0.02
300	29	273	1.52	0.41	0.19	0.13	0.11	0.08	0.08
	30	266	1.49	0.49	0.20	0.17	0.11	0.16	0.11
	31	387	0.95	0.67	0.35	0.27	0.15	0.11	0.15
	32	277	1.53	0.65	0.25	0.22	0.13	0.08	0.13
Mean			1.37	0.56	0.25	0.20	0.13	0.11	0.12
SE			0.14	0.06	0.04	0.03	0.01	0.02	0.01
CMC	33	288	0.94	0.60	0.36	0.25	0.14	0.11	0.09
	34	410	1.14	0.39	0.43	0.27	0.22	0.18	0.16
	35	302	1.15	0.78	0.34	0.20	0.15	0.13	0.13
	36	281	0.91	0.57	0.33	0.19	0.12	0.11	0.08
Mean			1.04	0.59	0.37	0.23	0.16	0.13	0.12
SE			0.06	0.08	0.02	0.02	0.02	0.02	0.02

Table A-14

Fecal Excretion Rate (µg/hr) of Gold After Administration of ^{198}Au-ATM (1.0 mg Au/kg,im) in Rats Given ANIT in Carboxymethyl Cellulose 24 hr Beforehand.

Dose (mg/kg ANIT)	Rat	Weight (g)	Mid-time of Each Collection Period (hr)						
			12	36	60	84	108	132	156
150	25	280	0.03	0.09	0.06	0.10	0.09	0.07	0.06
	26	297	0.00	0.07	0.29	0.21	0.13	0.10	0.07
	27	315	0.03	0.34	0.18	0.21	0.16	0.11	0.09
	28	259	0.00	0.38	0.10	0.16	0.15	0.12	0.07
Mean			0.02	0.22	0.16	0.17	0.13	0.10	0.07
SE			0.01	0.08	0.05	0.03	0.02	0.01	0.01
300	29	273	0.14	0.19	0.09	0.08	0.09	0.06	0.06
	30	266	0.03	0.17	0.08	0.11	0.11	0.12	0.07
	31	387	0.00	0.00	0.00	0.45	0.08	0.08	0.15
	32	277	0.10	0.10	0.14	0.09	0.08	0.05	0.07
Mean			0.07	0.12	0.08	0.18	0.09	0.08	0.09
SE			0.03	0.04	0.03	0.09	0.01	0.01	0.02
CMC	33	288	0.16	0.27	0.16	0.15	0.09	0.10	0.06
	34	410	0.13	0.20	0.26	0.18	0.14	0.13	0.10
	35	302	0.27	0.24	0.19	0.14	0.09	0.08	0.06
	36	281	0.13	0.24	0.19	0.12	0.10	0.07	0.05
Mean			0.17	0.24	0.20	0.15	0.10	0.09	0.07
SE			0.03	0.01	0.02	0.01	0.01	0.01	0.01

CURRICULUM VITAE

EDUCATION:

Degrees	Year	Field	Institution
B.Sc. with Distinction	1978	Pharmacy	University of Alberta

AWARDS:

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RELATED WORK EXPERIENCE:

1978-present:	Licensed Pharmacist (APhA)
1979-1981:	Graduate Teaching Assistant (GTA)
1978-1983:	Research Training (M.Sc. program)
1974-1978:	Training for B.Sc. Pharmacy

PAPERS:

Biggs DF, Boland DM, Davis P, Wakaruk J:
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